FORMULATION AND EVALUATION OF IMMEDIATE RELEASE BILAYER TABLETS OF ANTI RETROVIRAL DRUGS

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IN

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Submitted by

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CERTIFICATE

*This is to certify that this dissertation thesis entitled "***FORMULATION AND EVALUATION OF IMMEDIATE RELEASE BILAYER TABLETS OF ANTIRETROVIRAL DRUGS"** *is a bonafide genuine research work carried out by* ***Mr. ELDHO MATHEW (Reg. No. 26106305)*** *in Partial fulfillment of the requirements for the award of degree in* ***Master of Pharmacy in Pharmaceutics,*** *of* ***The Tamilnadu Dr. M.G.R. Medical University, Chennai****, in the Research and Development Centre,* ***Micro Advanced Research Centre, Bangalore,*** *under my guidance and supervision to my fullest satisfaction.*

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DECLARATION BY CANDIDATE

It gives me great pleasure and satisfaction to declare that the dissertation entitled ***"FORMULATION AND EVALUATION OF IMMEDIATE RELEASE BILAYER TABLETS OF ANTI- RETROVIRAL DRUGS"*** *is a bonafide genuine research work carried out by me in the Research and Development Centre, Micro Advanced Research Centre, Bangalore, under the guidance* ***of Mrs. Ramya, (****Institutional Guide****)*** *Asst. Professor, Dept. of Pharmaceutics, R.V.S College of Pharmaceutical Sciences, Sulur, Coimbatore and* ***Mr. Rajesh Kshirsagar****,* (*Industrial Guide****)*** *Exe. Vice President of Micro Advanced Research Centre Bangalore.*

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LIST OF ABBREVIATIONS

API	-	Active Pharmaceutical Ingredient
AUC	-	Area under Curve
BP	-	British Pharmacopoeia
CCS	-	Croscarmellose Sodium
SSG	-	Sodium starch glycolate
CR	-	Controlled Release
CU	-	Content Uniformity
GMP	-	Good Manufacturing Practices
IH	-	In house
IR	-	Immediate release
HPLC	-	High Performance Liquid Chromatography
JP	-	Japanese Pharmacopoeia
IP	-	Indian Pharmacopoeia
Kp	-	Kilo pound
LMH	-	Lactose Monohydrate
MCC	-	Microcrystalline Cellulose
Mm	-	Millimetre
GI	-	Gastrointestinal
Mg	-	Milligram
Ml	-	Millilitre
Ph.Eur	-	European Pharmacopoeia
SD	-	Standard Deviation
USPNF	-	United States Pharmacopoeia National Formulary
%	-	Percentage
λmax	-	Maximum absorbance
°C	-	Degree centigrade
µg	-	Micrograms
FT-IR	-	Fourier Transformed-Infrared Spectroscopy
Hrs	-	Hours
Min	-	Minutes
I.V	-	Intravenous
LR	-	Laboratory reagent
PB	-	Phosphate buffer
RH	-	Relative Humidity
$t_{1/2}$	-	Elimination half life

UV	-	Ultra violet
CR	-	Controlled release
CU	-	Content uniformity
GIT	-	Gastro intestinal tract
GMP	-	Good Manufacturing Practices
MEC	-	Minimum effective concentration
DDS	-	Drug delivery system
PO	-	Physical observation
NRTI	-	Nucleoside reverse transcriptase inhibitors
NtRTIs	-	Nucleotide reverse transcriptase inhibitors
HIV	-	Human immunodeficiency virus
DNA	-	Deoxyribonucleic acid
HBV	-	Hepatitis B virus

1. ABSTRACT

The objective of this research work was to formulate, develop and evaluate combination of immediate release (IR) tablets of Tenofovir DF and Lamivudine. Tenofovir DF and Lamivudine are nucleotide and nucleoside reverse transcriptase inhibitor. These drugs have to be given in combination otherwise HIV virus develops resistance to these drugs.The tablets were prepared by dry granulation and wet granulation method. For the Immediate release formulation the disintegration time of the tablet must be optimised in order to have a faster release of drug in the dissolution profile. The disintegration time is managed by using the superdisintegrants like Croscarmellose sodium, sodium starch glycolate Type A in the formulation. The formulation trials were optimised by incorporating varying composition of Lactose monohydrate, microcrystalline cellulose as diluents, Croscarmellose sodium, Sodium starch glycolate Type-A as Superdisintegrants, Pregelatinized starch as binder, Magnesium stearate as lubricant. When the two drugs are combined the percentage release of the drugs was not matching with the innovator samples. So it was formulated as film coated immediate release bilayered tablets. The preformulation parameters such as bulk density, tapped density, compressibility index and hausner's ratio were analysed for prepared granules before compression. The thickness, hardness, friability, weight variation, disintegration time and drug content uniformity was evaluated for core and coated tablets. The In-Vitro drug release studied were performed in the USP Apparatus-II (Paddle) using 0.1N HCl as a dissolution media at 50rpm speed and temperature of 37°c ± 0.5°c. The % drug release at different time interval was estimated using UV method. Based on the evaluation result F9 trial was selected as the best formulation. These results indicated that the selected formulation was stable during the test period of accelerated stability studies. The In-vitro drug release profile of the drugs was compared with marketed reference products of Tenofovir DF and Lamivudine. All the evaluated result was found to be satisfied with the reference products.

2. INTRODUCTION

2.1 ORAL DRUG DELIVERY[1-5]

Oral drug delivery has been known for decades as the most widely utilized route of administration among all the routes that have been explored for the systemic delivery of drugs via various pharmaceutical products of different dosage forms. The reasons that the oral route achieved such popularity may be in part due to its ease of administration as well as the traditional belief that by oral administration the drug is well absorbed along with the gastrointestinal tract along with food stuff. It is the most desirable and preferred method of administering therapeutic agents for their systemic effects. In addition, the oral medication is generally considered as the first avenue investigated in the discovery and development of new drug entities and pharmaceutical formulations, mainly because of patient acceptance, convenience in administration, and cost-effective manufacturing process.

For many decades various pharmaceutical dosage form such as tablets, capsules, suppositories, creams, ointments, liquids, aerosols, and injectables have been used for the delivery of drugs to the patients for the treatment of various diseases. Even today conventional dosage forms are the primary pharmaceutical vehicles commonly seen in the prescription and over the counter drug market. The oral conventional types of dosage form are known to provide a prompt release of drug. The success of any technology relies on the ease of its manufacturing process and its desirable biopharmaceutical properties. The basic goal of drug therapy is to achieve a therapeutic effect. Almost 90% of all the drugs used to produce systemic effect are administered by oral route. Tableted drug delivery systems can range from relatively simple immediate release (IR) formulation to complex extended or modified release dosage forms. In any solid dosage forms, an important variable is the rate at which the active substance goes into solution or dissolves to reach the systemic circulation. Dissolution of the active substance is essential for it to be absorbed through the biological membranes into systemic circulation for eliciting its desired pharmacological activity. The most important role of a drug delivery system is to get the drug “delivered” to the site of action in sufficient amount and at the appropriate rate.

2.2 TABLETS[7-8]

Tablets may be defined as solid pharmaceutical dosage forms containing drug substances with or without suitable diluents and prepared either by compression or molding methods. They have been in widespread use since the latter part of the 19th century and their popularity continues.

Tablets remain popular as a dosage form because of the advantages, afforded both to the manufacturer [e.g.: simplicity & economy of preparation, stability and convenience in packing, shipping and dispensing] and the patient [e.g.: accuracy of dosage, compactness, post ability, blandness of taste and ease of administration].

Although tablets are more frequently discoid in shape, they also may be round, oval, oblong, cylindrical or triangular. They may differ greatly in size and weight depending on the amount of drug substance present and the intended method of administration.

2.2.1 Properties of Tablets:

The attributes of an acceptable tablet are as follows:

- The tablet must be sufficiently strong and resistance to shock and abrasion and to withstand handling, during manufacturing, packing, shipping, and use. Hardness and friability tests measure this property.
- Tablet must be uniform in weight and in drug content of the individual tablet. This is measured by the weight variation and content uniformity tests.
- The drug content of the tablet must show good bioavailability. This property is measured by the dissolution test. Accurate bioavailability can be obtained from the drug levels of the drug after its administration.
- Tablets must be elegant in appearance and must have characteristic shape, color, and other markings necessary to identify the product.
- Tablets must retain all these functional attributes, which include drug stability and efficacy.

2.2.2Advantages of Tablets:

- They are easy to administer.
- They are a unit dosage form, and they offer the greater capabilities of all oral dosage forms for the greatest dose precision and the least content variability.
- Their cost is lowest of all oral dosage forms.
- They are the lightest and most compact of all oral dosage forms.
- Product identification is potentially the simplest and cheapest, requiring no additional processing steps when employing an embossed or monogrammed punch face.
- They are in general the easiest and cheapest to package and ship of all oral dosage forms.
- They may provide the greatest ease of swallowing with the least tendency for "hang-up" above the stomach. Especially when coated, provided that tablet disintegration is not excessively rapid.
- They lend themselves to certain special release profile products, such as enteric or delayed release products.
- They are better suited to large-scale production than other unit oral forms.
- They have the best-combined properties of chemical, mechanical and microbiological stability of all the oral forms.
- One of the major advantages of tablet over capsules is that the tablet is essentially "tamperproof dosage form".

2.2.3Disadvantages of Tablets:

- Some drugs resist compression into dense compacts, owing to their amorphous nature or flocculent, low-density character.
- Drugs with poor wetting slow dissolution properties, intermediate to large dosages, optimum absorption high in the gastrointestinal tract or any combination of these features may be difficult or impossible to formulate and manufacture as a tablet that will still provide adequate or full drug bioavailability.
- Bitter tasting drugs, drugs with objectionable odor or drugs that are sensitive to oxygen or atmosphere moisture may require encapsulation or a special type of coating with may increase the most of the finished tablets.

2.2.4 Types of Tablets:

Tablets are classified according to their route of administration or function.

1. Tablets ingested orally

a) Compressed tablets
b) Multiple compressed tablets
c) Multilayered tablets
d) Sustained action tablets
e) Enteric coated tablets
f) Sugar coated tablets
g) Film coated tablets
h) Chewable tablets

2. Tablets used in the oral cavity

a) Buccal tablets
b) Sublingual tablets
c) Lozenge tablets and troches
d) Dental cones

3. Tablets administered by other routes

a) Implantation tablets
b) Vaginal tablets

4. Tablets used to prepare solutions

a) Effervescent tablets

5. Molded tablets or tablet triturates (TT)

a) Dispensing tablets (DT)
b) Hypodermic tablets (HT)

2.2.5 Tablet Manufacturing:

Tablets are compressed powders and their manufacturing is a complex, multistep process. The ultimate aim is to easily disperse in gastrointestinal fluid and complete absorption of API and at the same time, offer stability to the formulation.

The tablet manufacturing process can be broadly classified as:

1) Granulation method
    a. Wet granulation method
    b. Dry granulation method
2) Direct compression method

2.3 IMMEDIATE RELEASE TABLETS[6]

Pharmaceutical products designed for oral delivery and currently available on the prescription and over-the-counter markets are mostly the immediate release type, which are designed for immediate release of drug for rapid absorption.

Disintegrating agents are substances routinely included in tablet formulations and in some hard shell capsule formulations in order to promote moisture penetration and dispersion of the matrix of the dosage form in dissolution fluids. Superdisintegrants used to improve disintegrant efficiency resulting in decreased use levels when compared to traditional disintegrants.

Starch has been the disintegrant of choice in tablet formulation, and it is still widely used. For instance, starch generally has to be present at levels greater than 5% to adversely affect compatibility, especially in case of direct compression. Drug release from a solid dosage form can be promoted by addition of suitable disintegrants.

1.3.1 Definition:

The term immediate release pharmaceutical formulation includes any formulation in which the rate of release of drug from the formulation and the absorption of drug was neither appreciably nor intentionally retarded by galenic manipulations. Immediate release may be

provided along with an appropriate pharmaceutically acceptable diluent or carrier, in which diluent or carrier does not prolong the rate of drug release and absorption.

The term "release" includes the presentation of the drug from the formulation in to the GIT, to body tissues and into the systemic circulation. For GIT release, the release is under pH conditions such as pH=1-3. The formulation of invention may release at least 70% (preferably 80%) of active ingredient within 4 hours, such as within 3 hours, preferably 2 hours, more preferably within 1.5 hours, and especially within an hour (within 30 minutes) of administration, whether it may be oral or parenteral.

2.3.2 DESIRED CRITERIA FOR IMMEDIATE RELEASE DRUG DELIVERY SYSTEM:

Immediate release dosage form should-

1. In the case of solid dosage form it should dissolve or disintegrate in the stomach within a short period.
2. In the case of liquid dosage form it should be compatible with taste masking.
3. It should be portable without fragility concern.
4. Should have a pleasing mouth feel.
5. It shouldn't leave minimal or no residue in the mouth after oral administration.
6. Exhibit low sensitivity to environmental condition such as humidity and temperature.
7. It should be able to manufacture using conventional processing and packaging equipment at low cost.
8. Rapid dissolution and absorption of drug, which may produce quick onset of action.

2.3.3 ADVANTAGES OF IMMEDIATE RELEASE DRUG DELIVERY SYSTEM

An immediate release pharmaceutical preparation offers:

1. Improved compliance
2. Improved stability
3. Suitable for controlled or sustained release actives
4. Allows high drug loading
5. Ability to provide advantages of liquid medication in the form of solid preparation
6. Adaptable and amenable to existing processing and packaging machinery
7. Cost effective

2.3.4 EXCIPIENTS USED FOR IMMEDIATE RELEASE DOSAGE FORMS:

1. Bulking materials:

Bulking materials are essential in the formulation of fast melting tablets. The bulking material has the functions of a diluent, filler and cost reducer. Bulking agents improve the textural characteristics that in turn enhance the disintegration in the mouth, besides; adding bulk it also reduces the concentration of the active in the composition.

Eg. Mannitol, polydextrose, lactitol, DCL (direct compressible lactose) and starch hydrolystate for higher aqueous solubility and good sensory perception.

2. Emulsifying agents:

Emulsifying agents are important excipients for formulating immediate release tablets they helps in rapid disintegration and drug release. In addition, incorporating emulsifying agents is useful in stabilizing the immiscible blends and enhancing bioavailability.

Eg. Alkyl sulfates, propylene glycol esters, lecithin, sucrose and esters

3. Lubricants:

Lubricants, though not essential excipients, can further assist in making these tablets more palatable after they disintegrate in the mouth. Lubricants also remove grittiness and assist in the drug transport mechanism from the mouth down into the stomach.

Eg. Magnesium Stearate, Talc

4. Flavours and Sweeteners:

Flavours and taste-masking agents make the products more palatable and pleasing for patients. The addition of these ingredients helps in overcoming bitterness and undesirable tastes of some active ingredients. Both natural and synthetic flavours are used to improve the organoleptic characteristic of fast melting tablets. The addition of sweeteners contributes a pleasant taste as well as bulk to the composition.

Eg. Sugar, dextrose, fructose, aspartame, sodium saccharin, sugar alcohols and sucralose.

5. Superdisintegrants:

A disintegrant is an excipient, which is added to a tablet or capsule blend to aid in the breakup of the compacted mass when it is put into a fluid environment.

Advantages:

1. Effective in lower concentrations

2. Less effect on compressibility and flowability

3. More effective intragranularly

Mechanism of disintegrants

1) High swellability
2) Capillary action and high swellability
3) Chemical reaction

The most popular disintegrants are corn starch, soluble starch etc. which have been well dried and powdered. Starches have great affinity for water and swell when moistened thus facilitating the rupture of the tablet matrix, its disintegration action in tablets is due to capillary action. Spherical shape of starch increases the porosity of tablet thus promoting capillary action.

Classification of Super disintegrants may be organized into three classes based on their chemical structure. As shown in **Table 2.3.4.1**.

Table 2.3.4.1:- Classification of Superdisintegrants.

Structure Type (NF Name)	**Description**	**Trade Name**
1. Modified starches (Sodium starch glycolate NF)	Sodium carboxy methyl starch, the carboxymethyl groups induced hydrophilicity and cross-linking reduces solubility.	Explotab Primojel
2. Modified cellulose (**Croscarmellose NF**)	Sodium carboxy methyl cellulose which has been cross-linked to render the material insoluble.	Ac-Di-Sol Nymcel Solutab
3. Cross-linked polyvinylpyrrolidone (Crospovidone. NF)	Cross-linked polyvinylpyrrolidone, the high molecular weight and cross-linking render the material insoluble in water.	Crospovidone Kollidon Polyplasdone

2.4 BILAYERED TABLETS[9-10]

Bilayer tablets are composed of two or three layers of granulation compressed together. They have the appearance of a sandwich because the edges of each layer are exposed.

Advantages

- This dosage form has the advantage of separating two incompatible materials.
- The weight of each layer can be accurately controlled, in contrast to putting one drug of a combination product in a sugar coating.

- Two-layer tablets require fewer materials than compression coated tablets, weight less, and may be thinner.
- Monograms and other distinctive markings may be impressed on the surfaces of the multi-layer tablets.
- Analytical work may be simplified by separating of the layers prior to assay.
- Since there is no transfer to second set of punches and dies, as with the dry-coating machines, add shapes (such as triangles, squares, and ovals) present no operating problems except for those common tolling.

Bi-layer tablets are tablet, made by compressing two different granulations fed into a die succession, one on top of another, in layers. Each layer comes from a separate feed frame with individual weight control. Rotary tablet press can be set up for two or three layers. More are possible but the design becomes very special. **Figure 2.4.1** represents compression cycle of bi-layer tablet

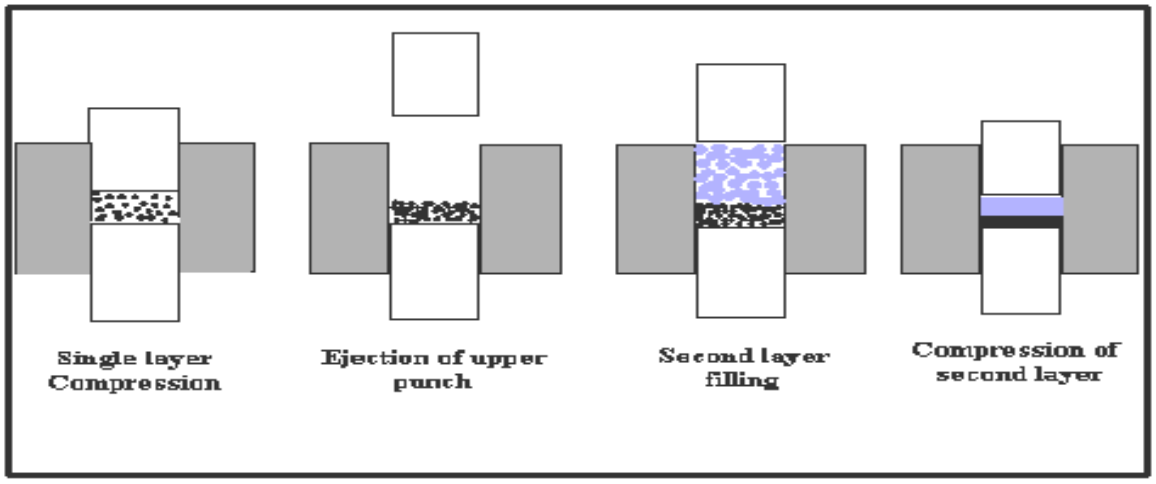


Figure 2.4.1:-Compression cycle of bi-layer tablet.

Steps of compression of bi-layer tablet:

1. Filling of first layer.
2. Compression of first layer.
3. Ejection of upper punch.
4. Filling of second layer.
5. Compression of both layer together.
6. Ejection of bi-layer tablet.

2.5 COATING PROCESS[11-13]:-

- **Tablet coating**

The application of coating to the tablets, which is an additional step in the manufacturing process, increases the cost of products; therefore, the decision to coat a tablet usually based on one or more of the following reasons.

- To mask the taste, odor or color of the tablets
- To provide physical and chemical stability to the drugs
- To control the release of the drug from the tablets
- To protect the drug from gastric environment of the stomach with an acid resistant enteric coating
- To incorporate another drug or formula adjuvant in the coating to avoid chemical incompatibilities or to provide sequential drug release
- To improve the pharmaceutical elegance by use of colors and contrast printing

- **Types of coating**

1. Enteric coating
2. Sugar coating
3. Film coating

2.5.1 Enteric coating

"An enteric coating is a coating system that resists disintegration or dissolution in gastric media but disintegrates or dissolves in intestinal fluid".

An enteric protected dosage form is the most common type of delayed release product. Enteric protection relies on the use of a polymeric material, usually as a coating which has pH selective solubility, taking advantage of change in pH that occurs as the dosage form progress through the gastro-intestinal tract.

In developing a drug delivery system for a particular drug candidate, formulation expertise to ensure the right choice of technology early in the development cycle by considering such factors as:

- Physico-chemical properties of the drug
- Physiology of the gastrointestinal tract and the manner in which the drug will be absorbed during passage
- Effect of food on the absorption rate and transit time of the drug.

➢ **Reason for enteric protection**

✓ Prevention of gastric irritation
  - e.g. Diclofenac sodium

✓ Protection of drug unstable in gastric fluid
  - e.g. Erythromycin

✓ Delivery of drug to intestinal site of action
  - E.g. Sulphasalazine

✓ Delivery of drug to best absorption site

✓ Delayed drug release
  - e.g. Once daily antihistamine

2.5.2 Sugar Coating

Sugar Coating is now rarely used in pharmaceuticals field because of it's Skillfull and tedious process.

There are various step of sugar coating

1. Seal Coating
2. Sub Coating
3. Syrup (Smoothing/Color) Coating
4. Polishing

2.5.3. Film Coating

Film Coating is widely used in pharmaceuticals to protect compressed tablets from **Light, Heat** and **Moisture**.

A very even application of the coating material is an important feature of the coating process. Coatings must be dense and without mechanical damage and cracks. Film coating is an effective process for the application of protective films for manipulating the product characteristics.

- **Film Coating Material**

There are following film coating material widely used in the formulation.

Table 2.5.3.1:-List of Film coating materials

Coating components	**Examples**
Film Former Polymer	Hydroxy Propyl Methyl Cellulose, Ethyl Cellulose, poly vinyl pyrollidone
Solvents	Water, ethanol, methanol, isopropanol, chloroform, acetone, methylethyl ketone, methylene chloride and more.
Plasticizers	Triethyl citrate, Triacetin, Dibutyl phthalate, Diethyl phthalate, Castor oil, polyethylene glycol 4000 and 6000.
Colorants	FDA approved colors for e.g. quinoline yellow lac, sunset yellow lac, iron oxide red.
Opaquant-extenders	Titanium dioxide, talc, aluminium silicate, magnesium carbonate, calcium sulfate, magnesium oxide and aluminium hydroxide.

- **Film defect**

Variation in formulation and processing conditions may results in unacceptable quality film coating. These defects are as follows.

- Sticking and Picking
- Roughness
- Orange peel effect
- Twin formation
- Bridging and Filling
- Hazing or dull film

2.6 EVALUATION OF TABLETS[6]

The tablets are subjected to the following quality control tests:

1. Weight variation
2. Friability
3. Hardness
4. Diameter
5. Length/ Width
6. Disintegration
7. In vitro Dissolution
8. Stability studies

Weight variation:

The weight variation test is carried out to ensure uniformity in the weight of tablets in a batch. The total weight of 20 tablets from each formulation was determined and the average weight was calculated. The individual weights of the tablets were also determined accurately and the weight variation was calculated.

Hardness:

The hardness of tablet is an indication about its strength. Here the force required to break the tablet is measured. The force is measured in kg and the hardness of about 3-5 kg/cm^2 is considered to be satisfactory for uncoated tablets. Hardness of 10 tablets from each formulation was determined by Monsanto hardness tester.

Friability test:

Friability is the loss in the weight of tablet in the container due to removal of fine particles from the surface. Friability test is carried out to find the ability of the tablet to withstand abrasion in packaging, handling and transport. Roche friabilator was employed for finding the friability of the tablets. 20 tablets from each formulation were weighed and placed in Roche friabilator that rotated at 25 rpm for 4 minutes. The tablets were

dedusted and weighed again. The percentage of weight loss was calculated again. The percentage of weight loss was calculated using the formula

% Friability = [(W1-W2)100]/W1

Where,

W1= Weight of tablet before test

W2 = Weight of tablet after test

Disintegration test:

The USP device to test disintegration is six glass tubes that are 3cm long, open at the top, and held against 10# screen at the bottom end of the basket rack assembly. One tablet is placed in each tube and the basket rack is positioned in 1 litre beaker of distilled water at 37± 2 ° C, such that the tablets remain below the surface of the liquid on their upward movement and descend not closer than 2.5cm from the bottom of the beaker.

IN VITRO DRUG RELEASE STUDIES

The immediate release tablets are subjected to in vitro drug release studies in 0.1N HCl for 30 minutes to access the ability of the formulation for providing immediate drug delivery.

Drug release studies were carried out in eight stage dissolution test apparatus using specified volume of dissolution media maintained at 37 ± 1^0c. The tablets are kept in the cylindrical basket and rotated at 100 rpm ,5ml of the sample from the dissolution medium are withdrawn at each time interval (2, 3, 5, 10, 15&30 minutes) and 5ml of fresh medium was replaced each time. The samples were filtered and from the filtrate 1ml was taken and diluted to 10ml.

In vitro dissolution kinetic studies

The drug release data were plotted and tested with zero order (Cumulative % drug released Vs time), First order (Log %Remained Vs time). The zero order release kinetics was shown in Figures. The First order release kinetics were shown in figures. The in vitro dissolution kinetic parameters, dissolution rate constants (K), correlation coefficient ®, the times (t50) for 50% drug released (half life) and dissolution efficiency were

calculated and presented in the tables of following chapters. From the slope of linear plots, the dissolution rates were calculated.

First – order release kinetics

$\log Q1 = \log Qo + k_1 t\ 2.303$

The First order equation describes that the release from systems is concentration dependent. Where Qo is the initial amount of the drug, t is in minutes and k_1 describes the dissolution rate constant for first order release kinetics. A plot of the logarithm of the percent drug remained against time will be linear if the release obeys first- order release kinetics. Values of release rate constant k_1 were obtained in each case from the slope of the log % drug remained versus time plots.

Dissolution efficiency

DE is defined as the area under the dissolution curve up to the time 't' expressed as a percentage of the area of the trapezoid described by 100% dissolution in the same time.

DE = t d y.dt Yidd.t

2.7 REVERSE-TRANSCRIPTASE INHIBITORSS[26]

2.7.1 INTRODUCTION

Reverse-transcriptase inhibitors (RTIs) are a class of antiretroviral drug that used to treat HIV infection, tumors and cancer. RTIs inhibit activity of reverse transcriptase, a viral DNA polymerase enzyme that retroviruses required to reproduce.

2.7.2 MECHANISM

When HIV infects a cell, reverse transcriptase copies the viral single stranded RNA genome into a double-stranded viral DNA. The viral DNA is then integrated into the host chromosomal DNA, which then allows host cellular process, such as transcription and translation to reproduce the virus. RTIs block reverse transcriptase's enzymatic function and prevent completion of synthesis of the double-stranded viral DNA, thus preventing HIV from multiplying.

A similar process occurs with other types of viruses. The hepatitis, for example, carries its genetic material in the form of DNA, and employs a RNA-dependent DNA polymerase to replicate. Some of the same compounds used as RTIs can also block HBV replication; when used in this way they are referred to as polymerase inhibitors.

2.7.3 TYPES

RTIs come in three forms:

- Nucleoside analog reverse-transcriptase inhibitors (NARTIs or NRTIs)
- Nucleotide analog reverse-transcriptase inhibitors (NtARTIs or NtRTIs)
- Non- nucleoside reverse-transcriptase inhibitors (NNRTIs)

The mode of action of NRTIs and NtRTIs is essentially the same; they are analogues of the naturally occurring deoxynucleotides required to synthesize the viral DNA and they compete with the natural deoxynucleotides for incorporation into the growing viral DNA chain. However, unlike the natural deoxynucleotides substrates, NRTIs and NtRTIs lack a 3'-hydroxyl group on the deoxyribose moiety. As a result, following incorporation of an NRTI or NtRTI, the next incoming deoxynucleotide cannot form the next 5'-3' phosphodiester bond needed to extend the DNA chain. Thus, when an NRTI or NtRTI is incorporated, viral DNA synthesis is halted, a process known as chain termination. All NRTIs and NtRTIs are classified as competitive substrate inhibitors.

In contrast, NNRTIs have a completely different mode of action. NNRTIs block reverse transcriptase by binding at a different site on the enzyme, compared to NRTIs and NtRTIs. NNRTIs are not incorporated into the viral DNA but instead inhibit the movement of protein domains of reverse transcriptase that are needed to carry out the process of DNA synthesis. NNRTIs are therefore classified as non-competitive inhibitors of reverse transcriptase.

2.7.3.1 NUCLEOSIDE ANALOG REVERSE-TRANSCRIPTASE INHIBITORS (NARTIS OR NRTIS)

Nucleoside analog reverse-transcriptase inhibitors (NARTIs or NRTIs) compose the first class of antiretroviral drugs developed. In order to be incorporated into the viral DNA,

NRTIs must be activated in the cell by the addition of three phosphate groups to their deoxyribose moiety, to form NRTI triphosphates. This phosphorylation step is carried out by cellular kinase enzymes.

Eg.Zidovudine, Didanosine, Zalcitabine, Stavudine, Lamivudine, Abacavir, Emtricitabine

Entecavir and Apricitabine

2.7.3.2 NUCLEOTIDE ANALOG REVERSE-TRANSCRIPTASE INHIBITORS (NTARTIS OR NTRTIS)

Normally, nucleoside analogs are converted into nucleotide analogs by the body. Taking nucleotide analog reverse-transcriptase inhibitors (NtARTIs or NtRTIs) directly allows conversion steps to be skipped.

Eg. Tenofovir, Adefovir

2.7.3.3 NON-NUCLEOSIDE REVERSE-TRANSCRIPTASE INHIBITORS (NNRTIS)

Non-nucleoside reverse-transcriptase inhibitors (NNRTIs) are the third class of antiretroviral drugs that were developed. In all cases, patents remain in force until beyond 2007.

Eg. Efavirenz, Nevirapine, Delavirdine, Etravirine

2.7.4 MECHANISMS OF RESISTANCE TO REVERSE-TRANSCRIPTASE INHIBITORS

While NRTIs and NNRTIs alike are effective at terminating DNA synthesis and HIV replication, HIV can and eventually does develop mechanisms that confer the virus resistance to the drugs. HIV-1 RT does not have proof-reading activity, this combined with selective pressure from the drug leads to mutations in reverse transcriptase that make the virus less susceptible to NRTIs and NNRTIs. Aspartate residues 110, 185, and 186 in the reverse transcriptase polymerase domain are important in the binding and incorporation of nucleotides. The side chains of residues K65, R72, and Q151 interact with the next incoming nucleotide. Also important is L74, which interacts with the template strand to position it for base pairing with the nucleotide. Mutation of these key amino acids results in reduced incorporation of the analogs.

2.7.4.1NRTI RESISTANCE

There are two major mechanisms of NRTI resistance. The first being reduced incorporation of the nucleotide analog into DNA over the normal nucleotide. This results from mutations in the N-terminal polymerase domain of the reverse transcriptase that reduce the enzyme's affinity or ability to bind to the drug. A prime example for this mechanism is the M184V mutation that confers resistance to lamivudine (3TC) and emtricitabine (FTC). Another well characterized set of mutations is the Q151M complex found in multi-drug resistant HIV which decreases reverse transcriptase's efficiency at incorporating NRTIs, but does not affect natural nucleotide incorporation. The complex includes Q151M mutation along with A62V, V75I, F77L, and F116Y. A virus with Q151M alone is intermediately resistant to zidovudine (AZT), didanosine (ddI), zalcitabine (ddC), stavudine (d4T), and slightly resistant to abacavir (ABC). A virus with Q151M complexed with the other four mutations becomes highly resistant to the above drugs, and is additionally resistant to lamivudine (3TC) and emtricitabine (FTC). The second mechanism is the excision or the hydrolytic removal of the incorporated drug or **pyrophosphorlysis**. This is a reverse of the polymerase reaction in which the pyrophosphate/PPI released during nucleotide incorporation reacts with the incorporated drug (monophosphate) resulting in the release of the triphosphate drug. This 'unblocks' the DNA chain, allowing it to be extended, and replication to continue. Excision enhancement mutations, typically M41L, D67N, K70R, L210W, T215Y/F, and K219E/Q, are selected for by thymidine analogs AZT and D4T; and are therefore called thymidine analog mutations (TAMs). Other mutations including insertions and deletions in the background of the above mutations also confer resistance via enhanced excision.

3.0 LITERATURE REVIEW

1. The United state pharmacopoeia (2009): USP reports the use of dissolution media such as 0.1N HCl as official media for drug Tenofovir DF and the maximum wave length was 260nm in their respective monographs[34].

2. B .Praveenreddy et al; (2011): studied on formulation and evaluation of immediate release efavirenz, Tenofovir DF and Lamivudine bilayer tablets by using 2% SLS as dissolution medium.Here the SLS in efavirenz layer is incompatible with Tenofovir DF. Here wet granulation method was used for granulation[16].

3. Syeed azeem et al; (2011): studied on the immediate release delivery system. According to him the basic approach in development of immediate tablets is the use of superdisintegrants like Cross linked carboxymethylcellulose, Sodium starch glycolate, Polyvinylpyrrolidone etc. which provide rapid disintegration of tablet after administration. A wide range of drugs can be considered candidates for this dosage form[6].

***4. Bytul M.Rahman et al*; (2008):** studied on effect of starch 1500 as a binder and disintegrant in Lamivudine tablets prepared by high shear wet granulation. Fully pregelatinized starch is currently being used as binder in wetgranulated formulations. But due to the gelatinization, much of the disintegration properties are lost. Partially pregelatinized starches (Starch 1500) have a mixture of properties of both native and fully gelatinized starches made them useful both as a binder and disintegrant in wet granulated formulations. Starch 1500 performed as an excellent binder producing a granulation that was compressible and produced Lamivudine tablets of improved hardness and friability compared with those prepared with povidone[17].

5. Varun Dasari et al; (2010): studied on formulation and evaluation of Lamivudine multiunit floating dosage forms using noval lipoidal polymers. Here he compared the dissolution characteristics of his optimized formulation with that of the pure drug and the marketed formulation[18].

6. Ramesh et al; (2010): studied on the formulation and evaluation of the Bi-Layered sustained release matrix tablets of Metformin HCl SR and Pioglitazone.The tablets were prepared using sodium carboxymethylcellulose(SCMC) and Hydroxypropyl Methyl cellulose (HPMC K4M & HPMC 15cps) as bio-adhesive polymers and croscarmellose sodium to act as an impermeable backing layer[19].

7. Shirkhedkar Atul A et al; (2009: studied on the application of UV-Spectrophotometric methods for estimation of Tenofovir DF in tablets. Here Tenofovir DF was estimated at 260nm in 0.1N HCl.It showed amplitude at 273nm[20].

8. Alexander Kuo et al; (2004): studied on Tenofovir DF for the treatment of Lamivudine resistant hepatitis B. The addition of Tenofovir DF to the existing regimen of Lamivudine resulted in a median decline of 4.5 log10 copies/mL in HBV DNA levels (range, 3.2–6.3 log10 copies/mL) after a median treatment duration of 12 months (range, 6–16 mo). No significant adverse events were encountered during treatment. In patients with Lamivudine -resistant hepatitis B, treatment with Tenofovir DF is well tolerated and results in significant virological, serological, and biochemical improvements without the complication of renal toxicity[29].

9. Prasanna A Nevase et al; (2011): studied on UV spectrophotometric method for estimation of Tenofovir Disoproxil fumarate tablet dosage form. They found a simple, rapid and accurate method for quantitative estimation of Tenofovir DF .They found that in methanol Tenofovir show maximum absorbance at 260nm[30].

***10. Defang et al;* (2005):** Formulate bi-layer tablet of Metformin and Glipizide. They conclude that extended release formulations exhibiting comparable i*n-vitro* release profiles using two formulation principles, i.e., elementary osmotic pump tablets and bi-layer hydrophilic matrix tablet[21].

11. Bhavesh Shiyani et al; (2008): Formulate bi-layer tablet of Metoclopramide Hydrochloride (MTH) and Ibuprofen (IB) for the effective treatment of migraine. Here MTH and IB were formulated as immediate release and sustained release layer respectively. MTH was formulated as immediate release layer by using various disintegrants like Ac.Di.Sol[22].

12. Althaf A.S. et al; (2010): prepared sustained release matrix tablets of Lamivudine to increase its bioavailability. The tablets were prepared by direct compression technique using polymers such as HPMC E15 & EC alone or in combination and other standard excipients. A factorial design was applied to systematically optimize the drug release profile. Optimized formulation shows release up to 16 hours as it fulfills all the requirements for sustained drug delivery system[23].

13. Prakash K. et al; (2007): formulate microcapsules of Lamivudine by using polymer such as CAP, CAB, EC & HPMCP to improve the release profile of drug. It is prepared by solvent evaporation technique. A factorial design was used to elucidate the effect of

variables viz. amount of drug release & the amount of polymer. The microcapsules were capapable for releasing drug more than 12 hrs & reducing frequency of administration[24].

14. ***Nayak B.S et al; (2009)****:* developed micro sphere of Lamivudine by using Modified solvent evaporation technique & enteric polymer such as Acryl coat, L30D & S100 to improv the release profile of drug. A factorial design was used to elucidate the effect of variables viz. amount of drug release & the amount of polymer. Plasma concentration was maintained above the minimum effective concentration for longer time after administration[25].

15. ***Tiwari A.K et al; (2011)****:* Formulated and evaluvated immediate release tablets of Drotaverine HCl. Wet granulation method was used because oof very high flow of powder blend that might create the problem of uneven dye filling. The superdisintegrant AC-Di-Sol and crospovidone were used for immediate release of drug from tablet[26].

16. ***Arkhel Alka et al; (2011)****:* Formulated and evaluated sustained release matrix tablet of Lamivudine using tamarind seed polysaccharide. The main aim of proposed is to focus on the possibilities of using polysaccharide in industries with particular reference to its physical, chemical properties for the formulation of new drug delivery systems[27].

17. ***Pranitha Yeluri et al; (2010)****:* Formulated and evaluated controlled release matrix tablet of Lamivudine using different proportion of guar gum as the retardant polymer and to study the effect of formulation factor such as polymer proportion on the invitro release of drug[28].

18. ***S. Tamizhrasi et al; (2009):*** Formulated and evaluated Lamivudine loaded polymethacrylic acid nanoparticles.The aim of the study was to prepare and evaluate polymethacrylic acid nanoparticles containing lamivudine in different drug to polymer ratio by nanoprecipitation method[31].

4. Aim and Objective

The concept of the immediate release tablets are formulated from past several years. The nucleoside and nucleotide analog reverse transcriptase inhibitors are the drugs used for HIV patients. These drugs should be given along with another nucleoside or nucleotide analog reverse transcriptase inhibitors. Single drug therapy is not suited because if the drug is giving as single dose for long term eventually the HIV virus develops resistance to the drugs. So the drugs have to be given in combination. Here the aim was to combine two Tenofovir DF and Lamivudine which are available in the market as single dose immediate release tablets. So the patients don't have to take two tablets. It will also reduce the cost of the tablet. Conventional method is (immediate release tablets) best method to formulate Tenofovir Df and Lamivudine tablets. The concept of immediate release attained by maintaining more than 50% drug loaded formulation by incorporating the superdisintegrants and binder, optimization of concentrations of those in the formulation to maintain faster disintegration time for faster drug dissolution profile.

- Tenofovir DF is a Nucleotide reverse transcriptase inhibitor, usually administered at doses of 300 mg per day.

- Lamivudine is a Nucleoside reverse transcriptase inhibitor, usually administered at doses of 300 mg per day.

- The pharmacokinetic and pharmacodynamics of the drug makes it suitable for administration through oral route

- The formulation of Tenofovir DF and Lamivudine in the form of oral tablets is a satisfactory tool to achieve its best therapeutic efficacy, since it is well absorbed and well tolerated through out the GI tract.

- The formulation of Tenofovir DF & Lamivudine bilayer film coated tablets improves its appearance and aids in good patient compliance. The immediate effect of these tablets enhances the onset time of action of the drug

Hence, the aim of the present work is to formulate immediate release film coated tablets of an Tenofovir DF and Lamivudine. The formula is optimized with various concentrations of

excipients and the method of preparation (Wet granulation) can be selected by performing pilot trial studies. The physical parameters evaluation, in-vitro drug release studies are conducted to justify the formulation efficacy.

4.1 Objective:

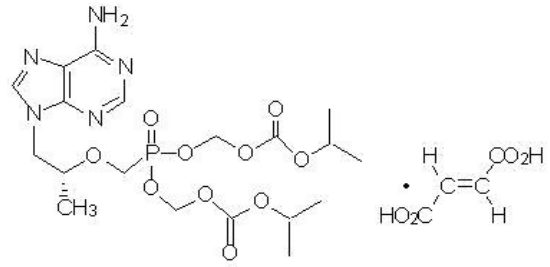
- Formulation and evaluation of combination of immediate release tablets of Tenofovir DF and Lamivudine.
- Improvement of the drug dissolution profile.
- To study the effect of super disintegrant concentration on the dissolution profiles of drug containing formulations.
- To study the effect of different granulation and formulation methods on dissolution profiles of drug.
- To determine the chemical compatibility of formulation containing various ratio of polymer and drug.

5. DRUG PROFILE

5.0 DRUG PROFILE OF TENOFOVIR DISOPROXIL FUMARATE[36-42]

- **DRUG NAME** : TENOFOVIR DISOPROXIL FUMARATE
- **CHEMICAL NAME** :Bis(1-methyl-ethyl)ester-(R)-5-[[2-(6-Amino-9H-purin- 9-yl)-1-methylethoxy]methyl]-2,4,6,8-tetraoxa-5-phosphanonanedioic acid 5-oxide(E)-2-butenedioate
- **CATEGORIES** : Anti-HIV Agents
- **BCS CLASSIFICATION** : Class-III
- **PHYSIOCOCHEMICAL PROPERTIES:**

Molecular Structure :



Appearance	: Tenofovir DF is white to off-white crystalline powder.
Solubility	: Soluble in water, slightly soluble in alcohol; sparingly soluble in methyl alcohol.
Formula	: $C_{19}H_{30}N_5O_{10}P \bullet C_4H_4O_4$
Mol.Mass	: 635.5 g/mol
Odour	:Odourless
Melting Point	:114°C-118°C
pH	:3.14 (1% aqueous solution)
pKa	:6.14 (0.1g in 60% of DD Water)
Log P	:0.78 (Organic Phase: Octanol, Aqueous phase: Water)
Cmax	: 0.3mcg.h/ml
Tmax	: 1 hour
Bioavliabilty	: 25%

❖ **MECHANISM OF ACTION:**

Tenofovir DF inhibits the activity of HIV reverse transcriptase by competing with the natural substrate deoxyadenosine 5'-triphosphate after incorporation into DNA, by DNA chain termination. Specifically, the drugs are analogues of the naturally occuring deoxynucleotides need to synthesize the viral DNA and they compete with the natural deoxynucleotides for incorporation into the growing viral DNA chain. However, unlike the natural deoxynucleotides substrates, NRTIs and NtRTIs (nucleoside/tide reverse transcriptase inhibitors) lack a 3'-hydroxyl group on the deoxyribose moiety. As a result, following incorporation of an NRTI or an NtRTI, the next incoming deoxynucleotide cannot form the next 5'-3' phosphodiester bond needed to extend the DNA chain. Thus, when an NRTI or NtRTI is incorporated, viral DNA synthesis is halted, a process known as chain termination. All NRTIs and NtRTIs are classified as competitive substrate inhibitors.

❖ **PHARMACOKINETICS:**

- **Absorption and Bioavailability:** The oral bioavailability in fasted patients is approximately 25%. Administration of food (high fat meal containing 40 to 50% fat) increases the oral bioavailability, with an increase in the AUC of approximately 40%.

- **Distribution:**

  1.3 ± 0.6 L/kg [Tenofovir DF 1.0 mg/kg]

  1.2 ± 0.4 L/kg [Tenofovir DF 3.0 mg/kg]

- **Metabolism:** Neither Tenofovir disoproxil nor Tenofovir are substrates of CYP450 enzymes.

- **Excretion** Renal (70% - 80%)

❖ **HALF LIFE :** Approximately 17 hours.

❖ **USES:**

Treatment of HIV-1 infection in adults and children 12 y of age and older, in combination with other antiretroviral agents; treatment of chronic hepatitis B infection in adults.

❖ **INDICATIONS:**

Tenofovir DF is a nucleotide analogue indicated in combination with other antiretroviral agents for the treatment of human immunodeficiency virus (HIV-1) infection.

❖ **DOSAGE AND ADMINISTRATION:**

- **Adults and Adolescents > 16 years of age**

The recommended oral dose of Tenofovir DF in HIV-1-infected adults and adolescents > 16 years of age is 300 mg daily orally without regard to food.

Pediatric Patients

The recommended oral dose of Tenofovir DF in HIV-1-infected pediatric patients 12 years of age and older with body weight greater than or equal to 35 kg (greater than or equal to 77 lb): The dose is one 300 mg Tenofovir DF tablet once daily taken orally, without regard to food.

❖ **SIDE EFFECTS:**

Tenofovir DF may cause lactic acidosis (a build-up of lactic acid in the body, which can be fatal). Lactic acidosis can start slowly and get worse over time. Get emergency medical help if you have even mild symptoms of lactic acidosis, such as: muscle pain or weakness, numb or cold feeling in your arms and legs, trouble breathing, stomach pain, nausea with vomiting, fast or uneven heart rate, dizziness, or feeling very weak or tired. Call your doctor at once if you have a serious side effect such as:

- liver damage - nausea, stomach pain, low fever, loss of appetite, dark urine, clay-colored stools, jaundice (yellowing of the skin or eyes);

- kidney problems - increased thirst and urination, loss of appetite, weakness, constipation, urinating less than usual or not at all;
- fever, chills, body aches, flu symptoms; or
- any other signs of new infection.

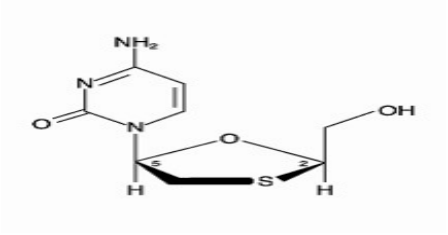
❖ **PRECAUTIONS:**

- Before taking Tenofovir DF, tell your doctor or pharmacist if you have allergy to it; or if you have any other allergies.
- Before taking this medication, tell your doctor or pharmacist about your medical history, especially of pancreatitis, kidney problems, liver problems and alcohol use.
- Avoid alcoholic beverages because they may increase the risk for liver problems and pancreatitis.
- This drug may make you dizzy. Do not drive, use machinery or any activity that requires alertness until you are sure that you can perform such activities safely.
- To decrease the risk of spreading HIV disease to others, always use an effective barrier method during all sexual activity.
- Care to be taken while using this drug in children because they may be more sensitive to the effects of the drug, especially the increased risk of pancreatitis.
- Kidney function declines as you grow older. This medication is removed by the kidneys. Avoid use in patients who have recently received nephrotoxic drugs.
- Tenofovir DF passes into breast milk. Because breast milk can transmit HIV, do not breast-feed.

5.1 DRUG PROFILE OF LAMIVUDINE[36-42]

- **DRUG NAME : LAMIVUDINE**
- **CHEMICAL NAME :**4-amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one
- **CATEGORIES :** Anti-HIV Agents
- **BCS CLASSIFICATION :** Class-III
- **PHYSIOCOCHEMICAL PROPERTIES:**

Molecular structure :



Appearance : White or almost white amourphos Powder

Solubility : Soluble in water, slightly soluble in alcohol; sparingly soluble in methyl alcohol

Formula : $C_8H_{11}N_3O_3S$

Mol Mass : 229.26 g/mol

Appearance : White to off white solid

Odour : Odourless

Melting Point : 172°C-178°C

pH : 7.01 (1% aqueous solution)

pKa : 9.48 (0.1g in 60% of DD Water)

Log P : 0.89 (Organic Phase: Octanol, Aqueous phase: Water)

Cmax : 1.28mcg.h/ml

Tmax **:** 0.5 to 2hour

Bioavliabilty **:** 87%

❖ **MECHANISM OF ACTION:**

Lamivudine is a synthetic nucleoside analogue and is phosphorylated intracellularly to its active 5'-triphosphate metabolite, Lamivudine triphosphate (L-TP). This nucleoside analogue is incorporated into viral DNA by HIV reverse transcriptase and HBV polymerase, resulting in DNA chain termination.

❖ **PHARMACOKINETICS:**

- **Absorption and Bioavailability:** Lamivudine was rapidly absorbed after oral administration in HIV-infected patients. Absolute bioavailability in adults is 86% ± 16% for the tablet and 87% ± 13% for the oral solution.

- **Distribution:** The apparent volume of distribution after IV administration of NL004 to 20 patients was 1.3 ± 0.4 L/kg, suggesting that Lamivudine distributes into extravascular spaces. Volume of distribution was independent of dose and did not correlate with body weight.

- **Metabolism:** The only detected metabolite of Lamivudine is trans-sulfoxide.

- **Elimination:** The primary routes of elimination of abacavir are metabolism by alcohol dehydrogenase to form the 5′-carboxylic acid and glucuronyl transferase to form the 5′-glucuronide. Lamivudine is excreted in human breast milk.

❖ **HALF LIFE** **:** 5 to 7 hours

❖ **USES:**

This drug is used with other medications to help control your HIV infection, thereby improving your quality of life. It may also lower your risk of complications from HIV (such as new infections, cancers). Lamivudine belongs to a class of drugs known as nucleoside reverse transcriptase inhibitors-NRTI.

Lamivudine is not a cure for HIV and it does not prevent the spread of HIV to others through sexual contact or blood contamination (such as sharing used needles).

❖ **OTHER USES:**

This drug may be used to prevent HIV infection after contact with the virus. A lower-strength Lamivudine E product is used for hepatitis B infection in people without HIV infection.

❖ **INDICATIONS:**

Lamivudine is a nucleoside analogue indicated in combination with other antiretroviral agents for the treatment of human immunodeficiency virus (HIV-1) infection. Limitation of use: The dosage of this product is for HIV-1 and not for HBV.

❖ **DOSAGE AND ADMINISTRATION:**

- **Adults and Adolescents > 16 years of age**

The recommended oral dose of Lamivudine in HIV-1-infected adults and adolescents > 16 years of age is 300 mg daily, administered as either 150 mg twice daily or 300 mg once daily, in combination with other antiretroviral agents. If Lamivudine is administered to a patient infected with HIV-1 and HBV, the dosage indicated for HIV-1 therapy should be used as part of an appropriate combination regimen

- **Pediatric Patients**

The recommended oral dose of Lamivudine Oral Solution in HIV-1-infected pediatric patients 3 months to 16 years of age is 4 mg/kg twice daily (up to. a maximum of 150 mg twice a day), administered in combination with other antiretroviral agents.

❖ **SIDE EFFECTS:**

Headache, dizziness, nausea, diarrhea, or trouble sleeping may occur. If any of these effects persist or worsen, notify your doctor or pharmacist promptly.

Changes in body fat (such as increased fat in the upper back and stomach areas, decreased fat in the arms and legs) may occur while you are taking HIV medication. The cause and long-term effects of these changes are unknown. Discuss the risks and benefits of therapy with your doctor, as well as the possible role of exercise to reduce this side effect.

A very serious allergic reaction to this drug is rare. However, seek immediate medical attention if you notice any symptoms of a serious allergic reaction, including: rash, itching/swelling (especially of the face/tongue/throat), severe dizziness, trouble breathing.

- **PRECAUTIONS:**
  - Before taking Lamivudine tell your doctor or pharmacist if you have any allergy to it; or if you have any other allergies.
  - Before using this medication, tell your doctor or pharmacist about your medical history, especially of: pancreatitis, kidney problems, liver problems, alcohol use.
  - Avoid alcoholic beverages because they may increase your risk for liver problems and pancreatitis.
  - This drug may make you dizzy. Do not drive, use machinery or do any activity that requires alertness until you are sure you can perform such activities safely.
  - To decrease your risk of spreading HIV disease to others, always use an effective barrier method during all sexual activity.
  - Care to be taken while using this drug in children because they may be more sensitive to the effects of the drug, especially the increased risk of pancreatitis.
  - Kidney function declines as you grow older. This medication is removed by the kidneys. . Avoid use in patients who have recently received nephrotoxic drugs.

- Lamivudine passes into breast milk. Because breast milk can transmit HIV, do not breast-feed.

6. EXCIPIENT PROFILE[34]

6.1 LACTOSE MONOHYDRATE

Non-proprietary names – lactose Monohydrate (BP), Lactosum monohydricum (Ph. Eur), Lactose (J.P), Lactose Monohydrate (USPNF).

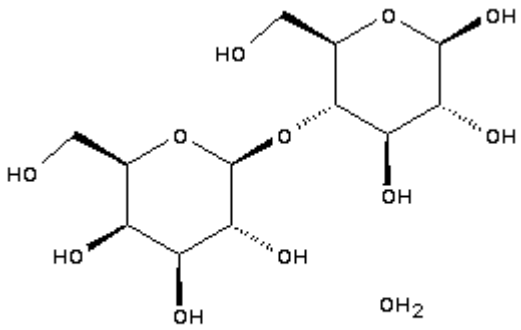
Synonym - Lactochem, Pharmatose, NF lactose, Capsulac, Prismalac, Sorbolac, Sachelac, Inhalac, Tablettose, Monohydrate lactose.

Chemical name: O-β-D-galactopyranosyl-(1-4)-β-D Glucopyranose anhydrous ,O-β-D-galactopyranosyl-(1-4)-β-D Glucopyranose monohydrate.

Empirical Formula - - $C_{12}H_{22}O_{11,}$ $C_{12}H_{22}O_{11}$. H_2O

Molecular weight – 342.30,360.31

Structural Formula –



lactose monohydrate

Functional category – binding agent, diluents for only powder inhalers, tablet binder, tablet and capsule diluents.

Applications in pharmaceutical formulations –

- Lactose monohydrate of various grades can be chosen for various applications.
- It is widely used as a filler or diluent in tabelts and in capsules
- It is also used in lyophilized products, to increase plug size and aid cohesion.
- Lactose in combination with sucrose (1:3) is to prepare sugar coating solutions.

- Direct compression grades are available as granulated/agglomerated and α-lactose monohydrate containing small amounts of anhydrous lactose.

Description – White to off-white crystalline particles of powder. It is odorless and slightly sweet tasting. The α-lactose is 20% sweet as sucrose; β lactose is 40% sweet as sucrose. Several different forms of lactose are commercially avaliable.

Incompatibilities – Condensation reactions are likely to occur between lactose and amino groups and incompatible with amino acids, aminophylline and lisinopril.

Safety - Adverse reactions to lactose are largely due to lactose intolerance, which occurs in individuals with a deficiency of the intestinal enzyme lactase.This results in lactose being undigested and may lead to clinical symptoms including abdominal cramps,diarrhea,distension and flatulence.

Storage/stability - Lactose develops a dark brown coloration on long storage, the reaction being accelerated by warm damp conditions. Mould growth may occur at humid conditions. It should be stored in a well closed container in a cool, dry place.

6.2 CROSCARMELLOSE SODIUM

Nonproprietary Names- BP: Croscarmellose sodium; PhEur: Carmellosum natricum conexum; USPNF: Croscarmellose sodium

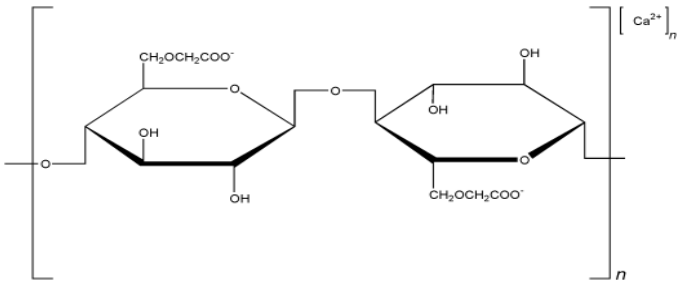
synonyms - Ac-Di-Sol; cross linked carboxymethylcellulose sodium; Explocel; modified cellulose gum; Nymcel ZSX; Pharmacel XL*;* Primellose; Solutab*;* Vivasol.

Functional Category -Tablet and capsule disintegrant.

Chemical name – Cellulose carboxymethyl ether calcium salt.

Empirical Formula and Molecular Weight - The USPNF 23 describes carboxymethylcellulose calcium as the calcium salt of polycarboxymethyl ether of cellulose.

Structural Formula:



Applications in Pharmaceutical Formulation or Technology:

- Used as a disintegrant for capsules, tablets, and granules.
- Croscarmellose sodium may be used in both direct-compression and wet-granulation processes in tablet formulations.
- Wicking and swelling ability of the disintegrant is best utilized when used in wet granulations.
- Used both the wet and dry stages of the process (intra and extra granularly).
- Croscarmellose sodium at concentrations up to 5% w/w may be used as a tablet disintegrant, 2% w/w is used in tablets prepared by direct compression and 3% w/w in tablets prepared by a wet-granulation process.

Table no 6.2.1 .Uses of Croscarmellose Sodium:

Use	**Concentration (%)**
Disintegrant in capsule	10-25
Disintegrant in tablets	0.5-5.0

Description: Croscarmellose sodium occurs as an odorless, white or greyish-white powder.

Incompatibilities

The efficacy of disintegrants, such as Croscarmellose sodium, may be slightly reduced in tablet formulations prepared by either the wet granulation or direct compression process that contain hygroscopic excipients such as sorbitol.

Croscarmellose sodium is not compatible with strong acids or with soluble salts of iron and some other metals such as aluminum, mercury and zinc.

Stability and Storage Conditions

Croscarmellose sodium is a stable though hygroscopic material. Croscarmellose sodium should be stored in a well closed container in a cool, dry place.

6.3 CELLULOSE, MICROCRYSTALLINE

Nonproprietary Names: BP: Microcrystalline cellulose; JP: Microcrystalline cellulose; PhEur: Cellulosum microcrystallinum; USPNF: Microcrystalline cellulose

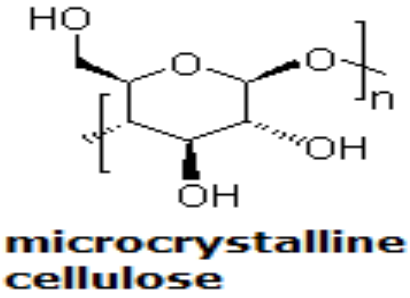
Synonyms: Avicel PH; **Celex**; cellulose gel; **Celphere**; **Ceolus KG**; crystalline cellulose; **E460**;Emocel; **Ethispheres**; ***Fibrocel***; **Pharmacel***;* **Tabulose***;* **Vivapur.**

Chemical Name - Cellulose [9004-34-6]

Empirical formula – $(C_6H_{10}O_5)_n$

Molecular weight – n=220 to 36000

Molecular structure –



microcrystalline cellulose

Functional Category: Adsorbent, Suspending agent, tablet and capsule diluents, tablet disintegrant.

Applications in Pharmaceutical Formulation or Technology

- Microcrystalline cellulose is widely used as a binder/diluent in oral tablet and capsule formulations where it is used in both wet-granulation and direct-compression processes.
- Also has some lubricant and disintegrant properties that make it useful in tableting.
- it is also used in cosmetics and food products

Table no 6.3.1: Uses of microcrystalline cellulose:

USE	CONCENTRATION (%)
Adsorbent	20-90
Anti-adherent	5-20
Capsule binder/diluent	20-90
Tablet disintegrants	5-15
Tablet binder/diluent	20-90

Description

Microcrystalline cellulose is purified, partially depolymerized cellulose that occurs as a white, odorless, tasteless, crystalline powder composed of porous particles. It is commercially available in different particle sizes and moisture grades that have different properties and applications.

Incompatibility – Microcrystalline Cellulose is incompatible with strong oxidizing agents.

Safety – Microcrystalline cellulose is widely used in oral pharmaceutical formulations and food products and is generally regarded as a relatively non-toxic and non-irritant material. Microcrystalline cellulose is not absorbed systemically following oral administration and thus has little toxic potential. Consumption of large quantities may have a laxative effect, although this is unlikely to be a problem when cellulose is used as excipient.

Stability/Storage conditions – Microcrystalline is stable though hygroscopic material. The bulk material should be closed in a well-closed container in a cool, dry place.

6.4 SODIUM STARCH GLYCOLATE

Nonproprietary Names

BP: Sodium starch glycolate

PhEur: Carboxymethylamylum natricum

USPNF: Sodium starch glycolate

Synonyms

Carboxymethyl starch, sodium salt; *Explosol*; *Explotab*; *Glycolys*; *Primojel*; starch carboxymethyl ether, sodium salt; *Tablo*; *Vivastar P*.

Chemical Name : Sodium carboxymethyl starch.

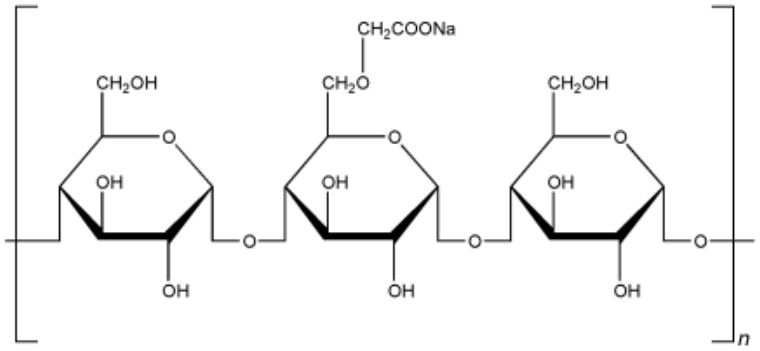
Empirical Formula and Molecular Weight

The USPNF 23 states that sodium starch glycolate is the sodium salt of a carboxymethyl ether of starch.

The PhEur 2005 describes three types of material: Types A and B occurs as the sodium salt of a cross-linked partly *O*-carboxymethylated potato starch, containing 2.8–4.2% and 2.0–3.4% of sodium respectively. Type C is the sodium salt of a cross-linked by physical dehydration, partly *O*-carboxymethylated starch containing 2.8–5.0% sodium.

Sodium starch glycolate may be characterized by the degree of substitution and crosslinking. The molecular weight is typically 5×10^5–1×10^6.

Structural Formula



Functional Category

Tablet and capsule disintegrant.

Applications in Pharmaceutical Formulation or Technology

- Sodium starch glycolate is widely used in oral pharmaceuticals as disintegrant in capsule and tablet formulations.
- It is commonly used in tablets prepared by either direct compression or wet granulation processes.
- The concentration employed in a formulation is between 2% and 8%, with the optimum concentration about 4%.
- Disintegration occurs by the rapid uptake of water followed by rapid and enormous swelling.
- By use of sodium starch glycolate, increasing the tablet compression pressure also appears to have no effect on disintegration time.
- Sodium starch glycolate can also be used as a suspending vehicle.

Description

Sodium starch glycolate is a white to off-white, odorless, tasteless, free flowing powder. It consists of oval or spherical granules, 30–100 μm in diameter, with some less spherical granules ranging from 10–35 μm in diameter.

Incompatibilities

Sodium starch glycolate is incompatible with ascorbic acid.

Stability and Storage Conditions

Tablets made with sodium starch glycolate have good storage properties. Sodium starch glycolate is stable. It should be stored in a well closed container in order to protect it from wide variations of humidity and temperature, which may leads to caking.

The physical properties of SSG remain unchanged for up to 3–5 years if it is stored at moderate temperatures and humidity.

6.5 PREGELATINISED STARCH

Nonproprietary Names

BP: Pregelatinised starch

PhEur: Amylum pregelificatum

USPNF: Pregelatinised starch

Synonyms

Compressible starch;instastarch; Lycatab C; Lycatab PGS; Merigel; National 78-1551; Pharma-Gel; Prejel; Sepistab ST 200; Spress B820; Starch 1500 G;Tablitz ; Unipure LD; Unipure WG220

Chemical Name : Pregelatinised starch

Empirical Formula and Molecular Weight

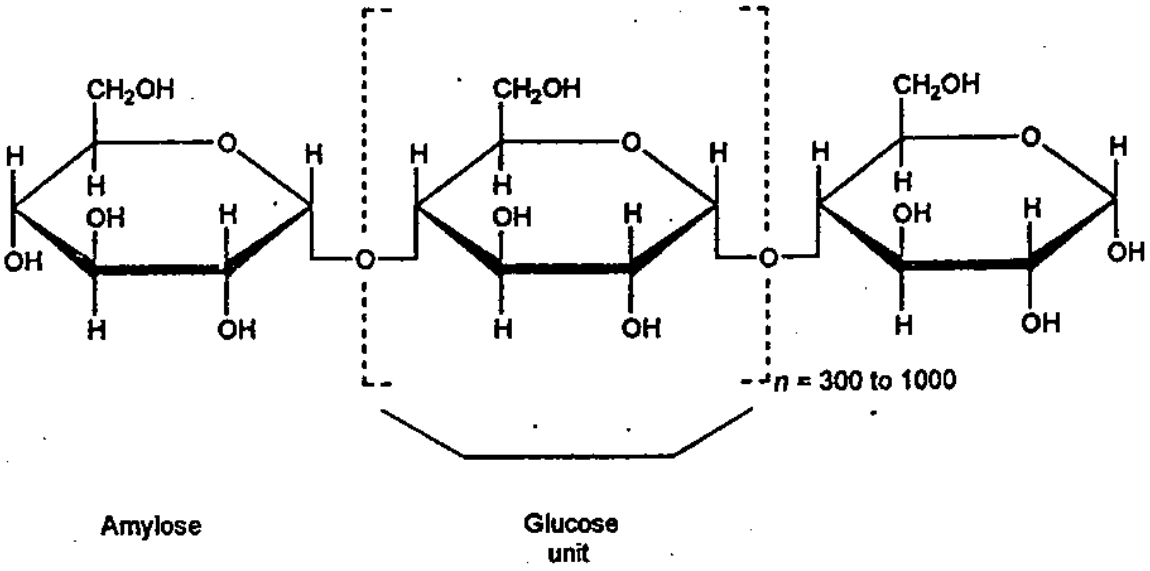
$(C_6H_{10}O_5)n$ where n=300-1000

Pregelatinised starch is a starch that has been chemically and/ or mechanically processed to rupture all or part of the starch granules and so render the starch flowable and directly compressible.

Typically pregelatinised starch contains 5% of free amylose , 15%of free amylopectin , and 80% unmodified starch.

The USPNF 20 does not specify the botanical orgin of the orginal starch , but the PhEur 2002 specifies that pregelatinized starch is obtained from maize (corn),potato, or rice starch.

Structural Formula



Functional Category

Tablet and capsule diluent; tablet and capsule disintegrant; tablet binder.

Applications in Pharmaceutical Formulation or Technology

- Pregelatinized starch is a modified starch used in oral capsule and tablet formulations as a binder , diluent, and disintegrant
- It is used as a tablet binder in dry- compression processes and in wet granulation processes

Table no 6.5.1. Uses of pregelatinized starch.

Use	Concentration (%)
Diluent (hard gelatin capsules)	5–75
Tablet binder (direct compression)	5–20
Tablet binder (wet granulation)	5–10
Tablet disintegrant	5–10

Description

Pregelatinized starch occurs as a moderately coarse to fine, white to off-white colored powder. It is odorless and has a slight characteristic taste.Examination of fully pregelatinized starch as a slurry in cold water, under a polarizing microscope, reveals no significant ungelatinized granules, i.e., no 'maltese crosses' characteristic of the starch birefringence pattern. Examination of samples suspended in glycerin shows characteristic forms depending upon the method of drying used during manufacture: either irregular chunks from drum drying or thin plates. Partially pregelatinized starch (e.g., Starch 1500G and Sepistab ST200) show retention of birefringence patterns typical of unmodified starch granules.

Incompatibilities

Stability and Storage Conditions

Pregelatinized starch is a stable but hygroscopic material, which has to be stored in a well closed container in a cool and dry place.

6.6 MAGNESIUM STEARATE

Nonproprietary Names

BP: Magnesium stearate

JP: Magnesium stearate

PhEur: Magnesii stearas

USPNF: Magnesium stearate

Synonym – Magnesium octadeconate, Octadecanoic acid, Magnesium salt, Stearic acid, magnesium salt.

Chemical name – Octadeconic acid magnesium salt.

Empirical formula – $C_{36}H_{70}MgO_4$.

Molecular weight- 591.34

Composition – The USPNF 23 describes magnesium stearate as a compound of magnesium with a mixture of solid organic acids that consists chiefly of variable proportions of magnesium stearate and magnesium palmitate ($C_{36}H_{70}MgO_4$). The Ph. Eur 2005 describes magnesium stearate as a mixture of magnesium salts of different fatty acids consisting mainly of stearic acid and in major proportions other fatty acids.

Structural Formula – $[CH_3(CH_2)_{16}COO]_2Mg$

Functional category – Tablet and capsule lubricant.

Pharmaceutical Applications –

- Magnesium stearate is widely used in cosmetics, foods and pharmaceutical formulatons.

- It is primarily used as a lubricant in capsule and tablet manufacture at concentrations between 0.25% and 5.0%.
- It is also used in barrier creams.

Description –

Magnesium stearate is a very fine, white, precipitated or milled, impalpable powder of low bulk density, having a faint odor of stearic acid and a characteristic taste. The powder is greasy to touch and readily adheres to the skin

Incompatibilities – Incompatible with strong acids, alkalis and iron salts. Avoid mixing with strong oxidizing materials. Magnesium stearate cannot be used in products containing aspirin, some vitamins and most alkaloidal salts.

Safety – Magnesium is generally regarded non-toxic following oral administration. However overconsumption of large quantities may cause laxative effect and mucosal irritation. No toxicity information is available relating to normal routes of occupational exposure.

Stability and storage conditions – Magnesium stearate is stable and should be stored in a well closed container in a cool and dry place.

7. INNOVATOR PRODUCT CHARACTERIZATION:

Table No.7.1 INNOVATOR PRODUCT CHARACTERIZATION OF TENOFOVIR DF[41-44]

Sr. No.	Particulars	Observation
1.	Generic name	Tenofovir Disoproxil Fumarate Tablets
2.	Brand name	VIREAD
3.	Storage condition	Store at 25°C excursions permitted to 15-30°C
4.	Embossing / Imprinting details	GILEAD and 4331 on one side and with 300 on other side.
5.	Dosage Form	Film coated Tablet
6.	Strength	300 mg
7.	Category	Antiretroviral drug
8.	Description	The tablets are almond-shaped, light blue, film coated and deboossed with GILEAD and 4331 on one side and with 300 on other side.
9.	Pack Details	Bottle with CRC cap of 30 tablets.
10.	Inactive Excipients	Croscarmellose sodium,Lactose monohydrate, magnesium stearate, microcrystalline cellulose, and pregelatinized starch. Opadry II Y-30-10671A, which contains FD&C blue #2 aluminium lake, hydroxypropyl methyl cellulose 2910, titanium dioxide and triacetin .
11.	Weight (mg)	693.0mg, 696.3mg, 681.6mg, 690.5mg
12.	Length (mm)	16.9mm, 16.92mm, 16.92mm
13.	Thickness (mm)	5.05mm, 5.08mm, 5.09mm
14.	Hardness (kp)	18kp, 19.3kp
15.	Disintegration Time	3min 02 sec to 3min 26sec

Table No.7.2 INNOVATOR PRODUCT CHARACTERIZATION OF LAMIVUDINE40-43

Sr. No.	Particulars	Observation
1.	Generic name	Lamivudine Tablets
2.	Brand name	EPIVIR
3.	Storage condition	Store at 25°C excursions permitted to 15-30°C
4.	Embossing / Imprinting details	Engraved with "GXEJ7" on one side.
5.	Dosage Form	Film coated Tablet
6.	Strength	300 mg
7.	Category	Antiretroviral drug
8.	Description	The tablets are grey modified diamond-shaped, film coated tablet engraved with "GXEJ7" on one side and plain on the reverse side.
9.	Pack Details	Bottle with CRC cap of 30 tablets.
10.	Inactive Excipients	Hydroxypropyl methyl cellulose, magnesium stearate, microcrystalline cellulose, poly ethylene glycol, polysorbate 80, sodium starch glycolate, titanium dioxide and black iron oxide.
11.	Weight (mg)	612.7mg, 615.2mg, 621.1mg
12.	Length (mm)	17.49mm, 17.62mm, 17.61mm
13.	Thickness (mm)	5.67mm, 5.64mm, 5.69mm
14.	Hardness (kp)	20.7kp, 23.2kp
15.	Disintegration Time	45 sec to 48 sec

8. PLAN OF WORK

- Preformulation study of API
- Preparation of stable Tenofovir DF and Lamivudine tablets 300mg/300mg.
- Preformulation studies.
- Optimization of concentration of excipients and method of manufacturing
- Coating of core tablets
- Evaluation of physical parameters like thickness, hardness, friability, and disintegration time of tablets.
- Evaluation of In-Vitro drug release of the tablets
- Comparative study of Tenofovir DF and Lamivudine tablets with innovator sample
- Selection of best formulation on the basis of In vitro drug release
- Stability Studies

9. MATERIALS AND EQUIPMENT:

Table no 9.1 List of Materials:

Name of Materials	Brand Name / Grade	Source
Tenofovir DF	IH	Hetero Labs
Lamivudine	IH	Hetero Labs
Croscarmellose sodium (primellose)	USPNF	DMV-FONTERRA
Lactose monohydrate (Pharmatose 200M)	USPNF	DMV-FONTERRA
Pregelatinized Starch (Starch 1500)	USPNF	COLORCON Asia Pvt Ltd
Sodium starch Glycolate Type A	USPNF	ROUQUETTE
Microcrystalline Cellulose (AvicelPH 102)	USPNF	FMC Biopolymer
Microcrystalline Cellulose (Avicel PH 101)	USPNF	FMC Biopolymer
Magnesium Stearate	USPNF	FERRO
Opadry White	IH	COLORCON Asia Pvt Ltd

Table no 9.2 List of Equipment:

Name of instrument	**Model no.**	**Make**
Electronic Weighing Balance (0.5 – 4100 g)	CPA8201	Sartorius
Analytical Balance (1 to 220g)	BT 2245	Sartorius
Octagonal/ Bin Blender	GMP LAB Model	Gansons
Rapid Mixer Granulator	PNEUMATIC	Sainath Bioler
Rapid Dryer	TG200	Retsch
Tap Density Tester USP	ETD-1020	Electrolab
Analytical Sieve Shaker	AS200	Retsch
Electronic Moisture Analyzer	MA 150	Sartorius
Tabletting Machine-8 Stn	MRT-8	KAMBERT
Bilayer Tableting machine-10stn	MRT-10	KARNAVATI
Disintegration Test Apparatus USP	ED-2L	Electrolab
Friabilator USP	EF-2	Electrolab
Vernier Calliper	CD-8" CSX	Mitutoyo Corporation
Homogenizer	RQ130	Remi Motors
Hardness Tester	8M	Dr.Schluniger
Mechanical Stirrer	RQT-124A	Remi Motors
Magnetic stirrer	5MLH	Remi Motors
Hot plate	H.P	Heat control
Pharma R&D Coater	Deluxe	Ideal Cures
Neocota	NEOCOTA - 5D	Neo machine
FTIR(Fourier Transform Infra Red Spectrophotometer)	IR Prestige-21	Shimadzu Emit Co. Ltd
PH meter	PH ME002	Eutech Instruments.
UV-Visible Spectrophotometer	UV-1700 Pharmaspec	Shimadzu Emit Co.LTD, Japan

10. Experimental work

10.1 Preformulation studies

10.1.1 Idenfication tests:

UV spectra: Diluted two drug samples in 0.1N HCl and UV spectrum of its obtained using a 1cm cell and scanning from 200 to 400nm. And compare the λ max value with the standard UV spectrum of Tenofovir DF & Lamivudine.

IR spectra: The FT-IR spectrum of pure Tenofovir DF & Lamivudine analyzed for study.

Solubility: Solubility analysis carried out in different solvents like water, HCl, Acetone, ethanol and methanol.

10.1.2 Drug exicipient compatibility studies:

Drug-Excipient compatibility studies form an important part of Preformulation studies. The interaction between the drug and excipients are determined after a specific time period by using suitable analytical technique like FTIR.

Procedure:

1) According to the functional category these excipients were mixed in different ratios with drug.

2) 1 gram of blend size was taken for the ratio calculation. These mixtures were kept in 40°C / 75 %RH.

3) After 4 weeks, the samples were withdrawn and analyzed with respect to Physical observation.

Table no 10.1.2 Details of Drug exicipient compatibility table:

Name of drug/excipients	Ratio	Test parameters		
		Initial	Period – 15 & 30 Days	
			40°C/75% RH	25°C/60% RH
Tenofovir DF	-	Physical observation	Physical observation	Physical observation
Lamivudine	-	Physical observation	Physical observation	Physical observation
Tenofovir DF +Lamivudine	-	Physical observation	Physical observation	Physical observation
Tenofovir DF +Lactose monohydrate(Pharmatose 200m)	1:2	Physical observation	Physical observation	Physical observation
Tenofovir DF + Microcrystalline Cellulose(Avicel PH101)	1:2	Physical observation	Physical observation	Physical observation
Tenofovir DF + Microcrystalline Cellulose(Avicel PH101)	1:2	Physical observation	Physical observation	Physical observation
Tenofovir DF + Pregelatinized Starch (Starch 1500)	1:0.5	Physical observation	Physical observation	Physical observation
Tenofovir DF+ Sodium starch glycolate-A	1:0.5	Physical observation	Physical observation	Physical observation
Tenofovir DF+ Croscarmellose sodium(Primellose)	1:0.5	Physical observation	Physical observation	Physical observation
Tenofovir DF+ Magnesium stearate	1:0.05	Physical observation	Physical observation	Physical observation
Tenofovir DF+ Opadry White	1:0.25	Physical observation	Physical observation	Physical observation
Lamivudine +Lactose monohydrate(Pharmatose 200m)	1:2	Physical observation	Physical observation	Physical observation

Lamivudine + Microcrystalline Cellulose(Avicel PH101)	1:2	Physical observation	Physical observation	Physical observation
Lamivudine + Microcrystalline Cellulose(Avicel PH101)	1:2	Physical observation	Physical observation	Physical observation
Lamivudine + Pregelatinized Starch (Starch 1500)	1:0.5	Physical observation	Physical observation	Physical observation
Lamivudine+ Sodium starch glycolate-A	1:0.5	Physical observation	Physical observation	Physical observation
Lamivudine+ Croscarmellose sodium(Primellose)	1:0.5	Physical observation	Physical observation	Physical observation
Lamivudine+ Magnesium stearate	1:0.05	Physical observation	Physical observation	Physical observation
Lamivudine+ Opadry White	1:0.25	Physical observation	Physical observation	Physical observation
Tenofovir DF+Lamivudine +Lactose monohydrate(Pharmatose 200m)	1:2	Physical observation	Physical observation	Physical observation
Tenofovir DF+Lamivudine + Microcrystalline Cellulose(Avicel PH101)	1:2	Physical observation	Physical observation	Physical observation
Tenofovir DF +Lamivudine + Microcrystalline Cellulose(Avicel PH101)	1:2	Physical observation	Physical observation	Physical observation
Tenofovir DF+Lamivudine + Pregelatinized Starch (Starch 1500)	1:0.5	Physical observation	Physical observation	Physical observation
Tenofovir DF+Lamivudine + Sodium starch glycolate-	1:0.5	Physical observation	Physical observation	Physical observation

A				
Tenofovir DF+Lamivudine + Croscarmellose sodium(Primellose)	1:0.5	Physical observation	Physical observation	Physical observation
Tenofovir DF+Lamivudine + Magnesium stearate	1:0.05	Physical observation	Physical observation	Physical observation
Tenofovir DF+Lamivudine + Opadry White	1:0.25	Physical observation	Physical observation	Physical observation
Tenofovir DF+ Placebo	1:2	Physical observation	Physical observation	Physical observation
Lamivudine+ Placebo	1:2	Physical observation	Physical observation	Physical observation
Tenofovir DF+Lamivudine+ Placebo	1:2	Physical observation	Physical observation	Physical observation

10.1.3 Preparation of Standard curve:

Preparation of samples for Tenofovir DF[20]:

100mg of drug was accurately weighed and dissolved and diluted to 100 ml with 0.1N HCl in volumetric flask. This was the primary stock solution, contained concentration of 1000 μ g/ml. From this primary stock solution, 1ml was accurately pipetted out and transferred in to a 100 ml volumetric flask and volume was made up to 100 ml with Solvent 0.1N HCl which contained the concentration of 10 μ g/ml. This is the secondary stock solution.

From secondary stock solution aliquots equivalent to 1, 2, 3, 4, and 5 ml were pipette out in to a series of 10 ml volumetric flask and volume was made up to 10 ml with 01N HCl. The absorbance of these solutions was measured against the 01N HCl as blank at 260 nm using UV-Visible double beam spectrophotometer. Then a calibration curve was plotted taking concentration in μ g/ml on X-axis and absorbance on Y-axis.

Preparation of samples for Lamivudine:

100mg of drug was accurately weighed and dissolved and diluted to 100 ml with 0.1N HCl in volumetric flask. This was the primary stock solution, contained concentration of 1000 μ g/ml. From this primary stock solution, 1ml was accurately pipette out and transferred in to a 100 ml volumetric flask and volume was made up to 100 ml with Solvent 0.1N HCl which contained the concentration of 10 μ g/ml. This is the secondary stock solution.

From secondary stock solution aliquots equivalent to 1, 2, 3, 4, and 5 ml were pipette out in to a series of 10 ml volumetric flask and volume was made up to 10 ml with 0.1N HCl. The absorbance of these solutions was measured against the 0.1N HCl as blank at 270 nm using UV-Visible double beam spectrophotometer. Then a calibration curve was plotted taking concentration in μ g/ml on X-axis and absorbance on Y-axis.

10.1.4 Preformulation studies of blend:

Preformulation testing is an investigation of physical and chemical properties of a drug substance alone and when combined with excipients. It is the first step in the rational development of dosage forms.

Preformulation commences when a newly synthesized drug shows sufficient pharmacologic promise in animal models to warrant evaluation in man. These studies should focus on those physicochemical properties of the new compound that could affect drug performance and development of an efficacious dosage form. A thorough understanding of these properties may ultimately provide a rationale for formulation design, or support the need for molecular modification.

Objective*:* The overall objective of preformulation testing is to generate information useful to the formulation in developing stable and bioavailable dosage forms.

Scope: The use of preformulation parameters maximizes the chances in formulating an acceptable, safe, efficacious and stable product.

10.1.4.1 Angle of repose[45]:

The angle of repose is the maximum angle that the plane of powder makes with the horizontal surface on rotation. Angle of repose is helpful in assessment of flow properties of particles which could be further related to packing densities and mechanical arrangements of particles.

The angle of repose of granules was determined by the fixed funnel and free standing cone method. The accurately weighed granules were taken in a funnel. The height of the funnel was adjusted in such a manner that the tip of the funnel just touched the apex of the heap of the granules. The granules were allowed to flow through the funnel freely onto the surface. The diameter of the powder cone measured and angle of repose was calculated using the following equation.

$$\tan \theta = h/r$$

Where h = height of the powder heap

r = radius of the powder heap

θ = is the angle of repose.

Table 10.1.4.1 Significance of Angle of Repose

S. No.	Angle of repose	Flow property
1	<25	Excellent
2	25-30	Good
3	30-40	Passable
4	>40	Poor

10.1.4.2 Determination of Bulk Density and Tapped Density[45]:

Bulk Density Determination:

Bulk density of a compound varies substantially with the method of crystallization, milling or formulation. It is of great importance when one considers the size of a high – dose capsule product or the homogeneity of a low dose formulation in which there are large differences in drug and excipient densities. In addition to bulk density, it is frequently desirable to know the true density of a powder for computation of void volume or porosity of packed powder beds.

Weighed quantity of the powder (W) was taken in a graduated measuring cylinder and volume (V0) was measured and bulk density was calculated using formula,

Bulk density = weight of powder/ volume of powder

B.D = W/V_0 g/ml

Tapped Density Determination:

Weighed of powder was taken in a graduated cylinder and the volume was measured (V_0). The graduated cylinder was fixed in the tapped densitometer and tapped for 500,750 and 1250 times until the difference in the volume after consecutive tapping was less than 2%.the final reading was denoted by (Vf) the volume of blend was used to calculate the tapped density, hausner's ratio and Carr's index.

Tapped density =W/V_f g/ml

Where W = Weight of the powder

V_0 = Initial volume

V_f = final volume

10.1.4.3 Carr's Compressibility Index[45]:

An indirect method of measuring powder flow from bulk densities was developed by Carr. The percentage compressibility of a powder was a direct measure of the potential powder arch or bridge strength and stability. Carr's index of each formulation was calculated according to equation given below:

Carr's Compressibility Index (%) = [(TD-BD) X 100] / TD
Where, TD = Tapped density and BD = bulk density

Table no 10.1.4.3 Relationships between % Compressibility and Flowability:

Sr. No.	% Compressibility	Flowability
1	5 – 15	Excellent
2	12 – 16	Good
3	18 – 21	Fair to Passable
4	23 – 35	Poor
5	33 – 38	Very Poor
6	> 40	Extremely Poor

10.1.4.4 Hausner's Ratio[45]:

Hausner's Ratio indicates the flow properties of the powder and is measured by the ratio of tapped density to bulk density. It is the ratio of tapped density and bulk density. Hausner found that this ratio was related to inter particle friction and, as such, could be used to predict powder flow properties. Generally a value less than 1.25 indicates good flow properties, which is equivalent to 20% of Carr's index.

Hausner's Ratio = Tapped density/Bulk Density

Table no 10.1.4.4 Significance of Hausner's ratio

Sr. No.	Hausner's Ratio	Property
1	0- 1.2	Free flowing
2	1.2- 1.6	Cohesive powder

10.1.5 Formulation development:

10.1.5.1 Formulation design for Dry Granulation:

1. **Trial 1 (F1)**

Aim: To take a trial batch of combination of Tenofovir DF &Lamivudine by dry granulation method

Formula

Table no 10.1.5.1.1 Working Formula F1

Sr. No.	Ingredients	Grade	Rational use	Quantity (mg/tab).
1	Tenofovir DF	IH	API	300
2	Lamivudine	USP	API	300
3	Microcrystaline cellulose (Avicel PH101)	USP	Diluent	50
4	Croscarmellose Sodium	USP	Disintegrant	30
5	Magnesium Stearate	USP	Lubricant	10
Extragranular Materials				
6	Microcrystaline cellulose (Avicel PH102)	USP	Diluent	44
7	Croscarmellose Sodium	USP	Disintegrant	20
Lubrication				
8	Magnesium Stearate	USP	Lubricant	6.0

10.1.5.1 Steps involved in the Trial (F1) by Dry Granulation Method:

1) **Dispensing:** Carryout the dispensing of the active pharmaceutical ingredient and excipients.

2) **Sifting:** Sift Tenofovir DF through sieve no #20 and Lamivudine through no #40. Sift Microcrystalline Cellulose (Avicel PH101), Croscarmellose sodium (Primellose), through #40 sieve and Magnesium Stearate through no #60 sieve.

3) **Drymixing:** Mix the sifted material Tenofovir DF, Lamivudine, Avicel PH 101 and Croscarmellose Sodium for 10 minute in a blender. Add the sifted Magnesium Stearate to the above blend and mix for 5 minutes.
4) **Compaction:** Pass the above blend through roller compactor and collect the flakes.

5) **Milling :** Mill the flakes using multimil with 2.0 mm screen and sift through sieve no #20 and the retain again milled through 1.5 mm screen and retains pass through sieve no #20.

6) **Sifting of Extra Granular and Lubricating Material:** Sift Microcrystalline cellulose (Avicel PH 102) and Croscarmellose sodium through sieve no #40

7) **Prelubrication:** Sift the dried granules to the Octagonal Blender, add the microcrystalline cellulose (Avicel PH 102) and Croscarmellose sodium which are pass through sieve no. #40, and rotate the blender for 10 min at 15 rpm.

8) **Lubrication:** Weigh the Magnesium Stearate, pass it through sieve no. #60. Add this to the above prelubricated material and blend for 5 min at 15 rpm.

9) **Compression:** Compress the lubricated blend on D/B tooling compression machine by using 18´ 8 mm Standard Concave punch plain surface on both sides.

Coating Solution Preparation:

Film coating solution Preparation:

Add the Opadry-White to purified water under constant stirring with mechanical stirrer for 45 minutes

Coating: Load the core tablets into the coating pan and coat the tablets with film coating solution.

Coating parameter:

- % Of solid content: 10%
- % Of build up: 3%
- Equipment name: Pharma R&D cota
- Pan Speed: 15 to 20 rpm
- Inlet temperatures: 60 °C

2. **Trial 2 (F2)**

Aim: To take a trial batch similar to F1 in with increasing concentration of CCS .

3. **Trial 3 (F3)**

Aim: To take a trial batch in which the two API's are separately compacted, milled blended, mixed and compressed

Formula

Table no 10.1.5.1.2 Working Formula F3

Sr. No.	Ingredients	Grade	Rational use	Quantity (mg/tab)
1	Tenofovir DF	IH	API	300
2	Microcrystaline cellulose (Avicel PH101)	USP	Diluent	30
3	Croscarmellose Sodium	USP	Disintegrant	13
4	Magnesium Stearate	USP	Lubricant	5
5	Lamivudine	USP	API	300
6	Microcrystaline cellulose (Avicel PH101)	USP	Diluent	225
7	Sodium Starch Glycolate (Type A)	USP	Disintegrant	28
8	Magnesium Stearate	USP	Lubricant	3.0

Steps involved in the Trial 3 (F3) by Dry Granulation Method:

1.Dispensing : Carryout the dispensing of the active pharmaceutical ingredient and excipients in dispensing booth.

2.Sifting : Sift Tenofovir DF through sieve no #20 and Lamivudine through no #40. Sift Microcrystalline Cellulose (Avicel PH101), Sodium Starch Glycolate (Type A), Croscarmellose sodium (Primellose), through #40 sieve and Magnesium Stearate through no #60 sieve.

3.Drymixing : Mix the sifted material Tenofovir DF, Avicel PH 101 and Croscarmellose Sodium for 10 minute in a blender. Add the sifted Magnesium Stearate to the above blend and mix for 5 minutes. Mix the sifted material Lamivudine, Avicel PH 101 and Sodium Starch Glycolate for 10 minute in a blender. Add the sifted Magnesium Stearate to the above blend and mix for 5 minutes.

4.Compaction : Pass the above blend of Tenofovir DF through roller compactor and collect the flakes. Then pass the blend of Lamivudine through the roller compactor and collect the flakes.

5.Milling : Mill the flakes using multimil with 2.0 mm screen and sift through sieve no #20 and the retains again mill through 1.5 mm screen and retains pass through sieve no #20.

6. Mixing : Mix the granules of Tenofovir DF and Lamivudine for 5 miuntes.

7.Compression : Compress the lubricated blend on D/B tooling compression machine by using 13.5 mm round Standard Concave punch plain surface on both sides.

Coating Solution Preparation:

Film coating solution Preparation: Similar to trial F1

4. **Trial 4 (F4)**

Aim: To take a trial batch similar to F3 by adding MCC Avicel PH 102 and Magnesium Stearate as extra granular portion.

10.1.5.2 Formulation design for Wet Granulation:

5. **Trial 5 (F5)**

Aim: To take a trial batch by wet granulation method.

Formula

Table no9.1.5.2 Working Formula F5

Sr. No.	**Ingredients**	**Grade**	**Rational use**	**Quantity (mg/tab)**
1	Tenofovir DF	IH	API	300
2	Microcrystaline cellulose (Avicel PH101)	USP	Diluent	100
3	Croscarmellose Sodium	USP	Disintegrant	28
4	Purified Water	USP	Binder solution	q.s
5	Lamivudine	USP	API	300
6	Microcrystaline cellulose (Avicel PH101)	USP	Diluent	140
7	Sodium Starch Glycolate (Type A)	USP	Disintegrant	12
8	Purified Water		Binder solution	q.s
Extra granular materials				
9	Microcrystaline cellulose (Avicel PH101)	USP	Diluent	98
10	Croscarmellose Sodium	USP	Disintegrant	12
Lubrication				
11	Magnesium Stearate	USP	Lubricant	10

Steps involved in the Trial 5 (F3) by Wet Granulation Method:

1.Dispensing: Carryout the dispensing of the active pharmaceutical ingredient and excipients in dispensing booth.

2.Sifting: Sift Tenofovir DF through sieve no #20 and Lamivudine through no #40. Sift Microcrystalline Cellulose (Avicel PH101), Sodium Starch Glycolate (Type A), Croscarmellose sodium (Primellose), through #40 sieve and Magnesium Stearate through no #60 sieve.

3.Drymixing of Tenofovir DF: Mix the sifted material Tenofovir DF, MCC (Avicel) PH 101 and Croscarmellose Sodium for 10 minute in rapid mixing granulator.

4. Granulation: Granulate the blend using purified water as the binder soluton.

5. Drying: Dried the wet granules using fluidized bed dryer. Check the LOD of the granules.

6.Drymixing of Lamivudine: Mix the sifted material Lamivudine, MCC (Avicel) PH 101 and Sodium Starch Glycolate for 10 minute in rapid mixing granulator.

7. Granulation: Granulate the blend using purified water as the binder solution.

8. Drying: Dried the wet granules using fluidized bed dryer. Check the LOD of the granules.

9. Sifting of Extra Granular and Lubricating Material: Sift Microcrystalline cellulose (Avicel PH 102) and Croscarmellose sodium through sieve no #40

10.Prelubrication: Sift the dried granules to the Octagonal Blender, add the microcrystalline cellulose (Avicel PH 102) and Croscarmellose sodium which are pass through sieve no. #40, and rotate the blender for 10 min,

11.Lubrication: Weigh the Magnesium Stearate, pass it through sieve no. #60. Add this to the above prelubricated material and blend for 5 min.

6.Compression: Compress the lubricated blend on D/B tooling compression machine by using 19.4 ´ 9mm Capsule shaped punch plain on both sides.

Coating Solution Preparation:

Film coating solution Preparation: Similar to trial F1

6. **Trial 6 (F6)**

Aim: To take a trial batch similar to trial 5 (F5) with increased concentration of CCS and addition of Pregelatinized Starch and Lactose Monohydrate.

10.1.5.3 Formulation design for Bilayer Tablets by Wet Granulation:

7. **Trial 7 (F7)**

Aim: To take a trial batch by compressing the API's as bilayer tablets

Formula

Table no 10.1.5.3 Working Formula F7

Sr. No	Ingredients	Grade	Rational use	Quantity (mg/tab)
	Layer 1			
1	Tenofovir DF	IH	API	300
2	Lactose Monohydrate (Pharmatose 200M)	USP	Diluent	150
3	Microcrystaline cellulose (Avicel PH101)	USP	Diluent	73
4	Croscarmellose Sodium	USP	Disintegrant	13
5	Pregelatinized Starch	USP	Binder	33
6	Purified Water	USP	Binder solution	q.s
Extra granular materials				
7	Microcrystaline cellulose (Avicel PH101)	USP	Diluent	73
8	Croscarmellose Sodium	USP	Disintegrant	13
Lubrication				
9	Magnesium Stearate	USP	Lubricant	7
	Layer 2			

10	Lamivudine	USP	API	300
11	Microcrystaline cellulose (Avicel PH101)	USP	Diluent	150
12	Sodium Starch Glycolate (Type A)	USP	Disintegrant	9
13	Purified Water	USP	Binder solution	q.s
Extra granular materials				
14	Microcrystalline cellulose (Avicel PH101)	USP	Diluent	80
15	Sodium Starch Glycolate (Type A)	USP	Disintegrant	9
Lubrication				
16	Magnesium Stearate	USP	Lubricant	7

Steps involved in the Trial 7 (F7) by Wet Granulation Method:

1.Dispensing: Carryout the dispensing of the active pharmaceutical ingredient and excipients.

2.Sifting: Sift Tenofovir DF through sieve no #20 and Lamivudine through no #40. Sift Microcrystalline Cellulose (Avicel PH101), Sodium Starch Glycolate (Type A), Croscarmellose sodium (Primellose), through #40 sieve and Magnesium Stearate through no #60 sieve.

Layer 1

3.Drymixing of Tenofovir DF: Mix the sifted material Tenofovir DF, MCC (Avicel) PH 101 and Croscarmellose Sodium for 10 minute in rapid mixing granulator.

4. Granulation: Granulate the blend using purified water as the binder solution.

5. Drying: Dried the wet granules using fluidized bed dryer. Check the LOD of the granules.

6. Sifting of Extra Granular and Lubricating Material: Sift Microcrystalline cellulose (Avicel PH 102) and Croscarmellose sodium through sieve no #40

7.Prelubrication: Sift the dried granules to the Octagonal Blender, add the microcrystalline cellulose (Avicel PH 102) and Croscarmellose sodium which are pass through sieve no. #40, and rotate the blender for 10 min,

8.Lubrication: Weigh the Magnesium Stearate, pass it through sieve no. #60. Add this to the above prelubricated material and blend for 5 min.

Layer 2

9.Drymixing of Lamivudine: Mix the sifted material Lamivudine, MCC (Avicel) PH 101 and Sodium Starch Glycolate for 10 minute in rapid mixing granulator.

10. Granulation: Granulate the blend using purified water as the binder solution.

11. Drying: Dried the wet granules using fluidized bed dryer. Check the LOD of the granules.

12. Sifting of Extra Granular and Lubricating Material: Sift Microcrystalline cellulose (Avicel PH 102) and Croscarmellose sodium through sieve no #40

13.Prelubrication: Sift the dried granules to the Octagonal Blender, add the microcrystalline cellulose (Avicel PH 102) and Croscarmellose sodium which are pass through sieve no. #40, and rotate the blender for 10 min,

14.Lubrication: Weigh the Magnesium Stearate, pass it through sieve no. #60. Add this to the above prelubricated material and blend for 5 min.

15.Compression: Compress the lubricated blend on D/B tooling compression machine by using 19.8 ´ 10.2mm Capsule shaped punch embossing "I" surface on one side and "49" on other side.

Coating Solution Preparation:

Film coating solution Preparation: Similar to trial F1

8. **Trial 8 (F8)**

   Aim: To take a trial batch similar to Trial F7 with increased concentration of superdisintegrants

9. **Trial 9 (F9)**

Aim: To take a trial batch similar to Trial F8 with increased concentration of superdisintegrants.

10. **Trial 10 (F10)**

Aim: To take a trial batch similar to Trial F9 in which the size of Tenofovir DF reduced by micronization process.

11. **Trial 11 (F11)**

Aim: To take a trial batch similar to Trial F10 in which the concentration of Microcrystalline cellulose (Avicel PH 101) is increased and the concentration of Pharmatose was reduced in Tenofovir DF part.

12. **Trial 12 (F12)**

Aim: To take a trial batch similar to Trial F10 by increasing the concentration of CCS and include the Pregelatinized Starch in the extragranular part of Tenofovir DF layer.

10.2 Formulation design:

Table no: 10.2.1 Formulation design for Dry Granulation

S.N	Ingredients	F1	F2	F3	F4
1	Tenofovir DF	300	300	300	300
2	Lamivudine	300	300	300	300
3	MCC(Avicel PH101 of layer 1)	50	25	30	145
4	MCC(Avicel PH101 of layer 2)	-	-	225	30
5	CCS	30	55	13	13
6	SSG type-A	-	-	28	28
7	Magnesium Stearate of layer 1	10	10	5	5
8	Magnesium Stearate of layer 2	-	-	3	3
Extragranular materials					
9	Avicel PH102	44	20	-	73
10	CCS	20	45	-	-
11	Magnesium Stearate	10	5	-	3
12	Total wt	760	760	904	900

Table no: 10.2.2 Formulation design for Wet Granulation:

All above values are taken in mg

S.N	Ingredients	F5	F6	F7	F8	F9	F10	F11	F12
Tenofovir DF Layer									
1	Tenofovir DF	300	300	300	300	300	300	300	300
2	Pharmatose 200m	-	151	153	153	153	153	60	142
3	MCC (Avicel PH101)	100	66	73	66	60	60	153	60
4	CCS	28	28	13	20	26	26	26	30
5	Starch 1500	-	33	33	33	33	33	33	-
Extragranular part									
6	MCC (Avicel PH102)	-		73	66	60	60	60	75
7	CCS	-		13	20	26	26	26	30
8	Starch 1500	-	-	-	-	-	-	-	15
Lubrication									
9	Magnesium Stearate	-	-	7	7	7	7	7	7
Lamivudine Layer									
1	Lamivudine	300	300	300	300	300	300	300	300
2	MCC (Avicel PH101)	140	150	155	150	145	145	145	145
3	SSG type-A	12	14	9	14	20	20	20	20
Extragranular Part									
4	MCC (AvicelPH102)	-	-	80	75	68	68	68	68
5	SSG type-A	-	-	9	14	20	20	20	20
Lubrication									
6	Magnesium Stearate	-	-	7	7	7	7	7	-
Extragranular Part of Layer 1+Layer 2									
7	MCC (Avicel PH102)	98	130	-	-	-	-	-	-
8	CCS	12	20	-	-	-	-	-	-
9	SSG	-	14	-	-	-	-	-	-
Lubrication of Layer 1+Layer 2									
10	Magnesium. Stearate	10	15	-	-	-	-	-	-
11	Total wt	1000	1220	1225	1224	1225	1225	1225	1225

10.3.1 Physical parameters of the uncoated tablet of optimized formula:

- Tablet weight : 1225 mg ± 3%
- Thickness : 7.10mm ± 0.10 mm
- Hardness :15 to 20 Kp
- Friability : Not more than 1%
- Disintegration time : Not more than 15 min

10.3.2 Physical parameters of the coated tablet of optimized formula:

- Tablet weight : 1265 mg ± 5%
- Thickness : 7.40mm ± 0.10 mm
- Disintegration time : Not more than 15 min

10.4 EVALUATION OF TABLETS[6]:-

10.4.1 Hardness test:

Tablet requires a certain amount of strength or hardness and resistance to friability to withstand mechanical shock of handling in manufacture, packing and shipping. Hardness tester measured the hardness of tablet. Five tablets from each batch were used for hardness studies and results were expressed in Kilo Pascals.

10.4.2 Thickness and diameter:

The thickness and diameter of tablets was carried out using verniercaliper. Five tablets were used for the above test from each batch results were expressed in millimeter.

10.4.3 Weight variation test:

Twenty tablets were selected at random, individually weighed in a single pan electronic balance and the average weight was calculated. The uniformity of weight was calculated. The uniformity of weight was determined according to I.P. specification. As per U.S.P not more than two of individual weight should deviate from average weight by more than 5% and none deviate more than twice that percentage.

10.4.4 Friability test:

It was done in Roche friabilator apparatus where the tablets were subjected to the combined effect of abrasion and shock by utilizing a plastic chamber that revolve at 25 rpm dropping the tablets at a distance of six inches with each revolution. Preweighed samples of 20 tablets were placed in the friabilator, which is then operated for 100 revolutions. The tablets were then dusted and reweighed. Conventional compressed tablets that loss less than 0.5 to 1.0% of their weight are generally considered acceptable.

$$\% F = \{1-(Wt/W)\} \times 100$$

where % F = Friability in percentage

W = Initial weight of tablets

Wt = Weight of tablets after revolution

10.5 TENTATIVE METHOD FOR ASSAY (UV METHOD)[11]:

Sample preparation of Tenofovir DF:

Weigh and finely powder not less than 20 tablets. Transfer an accurately weighed powder equivalent to about 100 mg of Tenofovir DF to a 100ml volumetric flask, add 30ml 0.1N HCl and shake for about 10 min. Make volume up to the mark with 0.1N HCl. Filter the solution with 0.45µm Whatman filter paper. Discard first 3 to 4ml of filtrate and pipette out 1ml of this filtrate to a 100 ml of volumetric flask and dilute up to the mark with diluents.

Standard preparation of Tenofovir DF:

Weigh accurately 100 mg of Tenofovir DF and transfer in to a 100ml volumetric flask, add 20 ml of and Shake for about 5 min. Add diluents and shake mechanically for 60 min. Make volume up to the mark with diluents(0.1N HCl).

Procedure:

Take the absorbance at 260 nm using 0.1N HCl as a blank for a background correction. Take absorbance in a triplicate of a standard solution and duplicate of a sample preparation.

Sample preparation of Lamivudine:

Weigh and finely powder not less than 20 tablets. Transfer an accurately weighed powder equivalent to about 100 mg of Lamivudine to a 100ml volumetric flask, add 30ml 0.1N HCl and shake for about 10 min. make volume up to the mark with 0.1N HCl. Filter the solution with 0.45µm Whatman filter paper. Discard first 3 to 4ml of filtrate and pipette out 1ml of this filtrate to a 100 ml of volumetric flask and dilute up to the mark with diluents.

Standard preparation of Lamivudine:

Weigh accurately 100 mg of Lamivudine and transfer in to a 100ml volumetric flask, add 20 ml of 0.1N HCl and Shake for about 5 min. add diluents and shake mechanically for 60 min. make volume up to the mark with diluents(0.1N HCl).

Procedure:

Take the absorbance at 270 nm using 0.1N HCl as a blank for a background correction. Take absorbance in a triplicate of a standard solution and duplicate of a sample preparation.

10.6 Dissolution studies (UV METHOD) [20]

In vitro dissolution studies of Tenofovir DF:

The release rate of Tenofovir DF from tablets was determined. The dissolution test was performed using United States Pharmacopoeia (USP) type II (paddle) apparatus,1000 ml of 0.1

N HCl at 37 ± 0.5°C and 50 rpm. A sample (10 ml) of the solution was withdrawn from the dissolution apparatus at the appropriate time for 5 min, and the samples were replaced with fresh dissolution medium. The samples were diluted into a suitable concentration with water with 0.1 N HCl. Absorbance of these solutions was measured at 260 nm using a Shimadzu UV/Visible double-beam spectrophotometer. Percentage drug release was calculated.

The drug content was calculated using the equation generated from standard calibration curve. The % drug release was calculated.

In vitro dissolution studies of Lamivudine:

The release rate of Lamivudine from tablets was determined. The dissolution test was performed using United States Pharmacopoeia (USP) type II (paddle) apparatus,1000 ml of 0.1 N HCl at 37 ± 0.5°C and 50 rpm. A sample (10 ml) of the solution was withdrawn from the dissolution apparatus at the appropriate time for 5 min, and the samples were replaced with fresh dissolution medium. The samples were diluted into a suitable concentration with water with 0.1 N HCl. Absorbance of these solutions was measured at 270 nm using a Shimadzu UV/Visible double-beam spectrophotometer. Percentage drug release was calculated.

The drug content was calculated using the equation generated from standard calibration curve. The % drug release was calculated.

Details of dissolution test:

- **Dissolution test apparatus** : USP II (Paddle)
- **Speed** : 50 rpm
- **Volume of medium** : 1000 ml
- **Time interval** : 5,10, 15, 20 , 30, 45, and 60
- **Medium used** : 0.1N HCl
- **Temperature** : 37 ± 0.5 ^{0}C

10.7 Data analysis:

To analyze the mechanism of release and release rate kinetics of the dosage form, the data obtained were fitted into Zero order, First order, Higuchi matrix, Peppas and Hixson Crowell model using PSP-DISSO – V2 software. Based on the r-value, the best-fit model was selected.

Zero order kinetics:

Drug dissolution from pharmaceutical dosage forms that do not disaggregate and release the drug slowly, assuming that the area does not change and No equilibrium conditions are obtained can be represented by the following equation,

$Q_t = Q_o + K_o t$

Where Q_t = amount of drug dissolved in time t.

Q_o = initial amount of the drug in the solution and

K_o = zero order release constant.

First order kinetics:

To study the first order release rate kinetics, the release rate data were fitted to the following equation,

$\text{Log } Q_t = \log Q_o + K_1 t/2.303$

Where Q_t is the amount of drug released in time t, Q_o is the initial amount of drug in the solution and K_1 is the first order release constant.

Higuchi model:

Higuchi developed several theoretical models to study the release of water soluble and low soluble drugs incorporated in semisolids and/or solid matrices. Mathematical expressions were obtained for drug particles dispersed in a uniform matrix behaving as the diffusion media. And the equation is,

$$Q_t = K_H \cdot t^{1/2}$$

Where

Q_t = amount of drug released in time t,

K_H = Higuchi dissolution constant.

Krosmeyer and Peppas release model:

To study this model the release rate data are fitted to the following equation,

$$Mt / M_{¥} = K \cdot t^{n}$$

Where $Mt / M_{¥}$ is the fraction of drug release, K is the release constant, t is the release time and n is the diffusion coefficient for the drug release that is dependent on the shape of the matrix dosage form

10.8 SIMILARITY FACTOR AND DISSIMILARITY FACTOR CALCULATION:

The similarity factor (f2) was defined by CDER, FDA, and EMEA as the "logarithmic reciprocal square root transformation of one plus the mean squared difference in percent dissolved between the test and reference release profiles".

Dissimilarity or difference factor (f1) describes the relative error between two dissolution profiles. It approximates the percent error between the curves. The percentage error is zero when the test and reference release profiles are identical and increases proportionally with dissimilarity between the two profiles.

There are several methods for dissolution profile comparison. f2 is the simplest among all those methods. Moore & Flanner proposed a model independent mathematical approach to compare the dissolution profile using two factors f1& f2.

$$f1 = \{ [\sum_{t=1}^{n} |R_t - T_t|] / [\sum_{t=1}^{n} R_t] \} . 100$$

$$f2 = 50. \log \{ [1 + (1/n) \sum_{t=1}^{n} (R_t - T_t)^2]^{-0.5} . 100\}$$

Where 'R_t 'and 'T_t' are the cumulative % dissolved at each of the selected 'n' time point of the reference & test product respectively. The factor f1 is proportional to the average difference between the two profiles, where as factor f2 is inversely proportional to the average

squared difference between the two profiles, with emphasis on the larger difference among all the time points. The similarity factor f2 and its significance are shown in the following table.

Table 10.8.1 Similarity factor f2 and its significance

S. No.	Similarity factor (f2)	Significance
1.	<50	Test and reference profiles are dissimilar.
2.	50 -100	Test and reference profiles are similar.
3.	100	Test and reference profiles are identical.
4.	>100	The equation yields a negative value.

10.9 STABILITY STUDY OF BI-LAYER TABLET[32-33]

The bi-layer tablets were stored at 40ºC ± 5ºC and 75% ± 5% relative humidity in stability chamber Samples were withdrawn at 1 month time intervals and evaluated for drug content, in-vitro drug release study, weight variation, hardness, thickness and friability.

11.1 PREFORMULATION STUDY

11.1.1 PREFORMULATION STUDIES OF TENOFOVIR DF:

API Charcterization-

Tenofovir DF was analyzed for various physical and analytical characterizations and was found to comply with the in house certificate of analysis of Hetero labs.

Physical Characterization of Tenofovir DF:

The results of physical characterization of the drug candidate are as follows-

1. Description

The received sample of Tenofovir DF was found to show the following characteristics and these are acceptable according to specification.

a) **Color:** White to off white**.**

b) **Nature:** Crystalline powder.

c) **Hygroscopic:** slightly hygroscopic

2. Solubility

The solubility of the Tenofovir DF was examined in various solvents. The results thus obtained were as follows-

Table No. 11.1.1.1 Solubility of drug sample

Sr.no	Solvent	Solubility
1	Water	Soluble
2	Acetone	Slightly soluble
3	Methanol	Sparingly soluble
4	Ethanol	Sparingly soluble

Table No. 11.1.1.2 *Other Test Performed*

Sr.no	Test	Specification	Result
1	Loss on Drying(at 75^{0}C)	Not more than 1.0%w/w	0.78% w/w
2	Assay:	Not less than 97.0% w/w and Not more than 101% w/w	99.5% w/w
3	Density i) Bulk ii) Tapped		 0.412 g/ml 0.636g/ml
4	Compressibility Index		35.22%
5	Hausner Ratio		1.543
6	Angle of Repose		32
7	Particle Size #	Not less than 98.0% should pass through #22 mesh	100 % passed through #22 mesh

Result: From above result of tests performed the all values are within the limits, it was concluded that the received sample of Tenofovir DF was pure and might be used in the formulation.

11.1.2 PREFORMULATION STUDIES of LAMIVUDINE:

API Charcterization-

Lamivudine was analyzed for various physical and analytical characterizations and was found to comply with the in house certificate of analysis of Hetero labs.

Physical Characterization of Lamivudine:

The results of physical characterization of the drug candidate are as follows-

1. **Description**

The received sample of Lamivudine was found to show the following characteristics and these are acceptable according to specification.

a) Color: White to off white**.**

b) Nature: Crystalline powder.

2. Solubility

The solubility of the Tenofovir DFwas examined in various solvents.The results thus obtained were as follows-

Table No. 11.1.2.1 Solubility of drug sample

Sr.no	Solvent	Solubility
1	Water	Soluble
2	Acetone	Slightly soluble
3	Methanol	Sparingly soluble
4	Ethanol	Sparingly soluble

Table No. 11.1.2.2 *Other Test Performed*

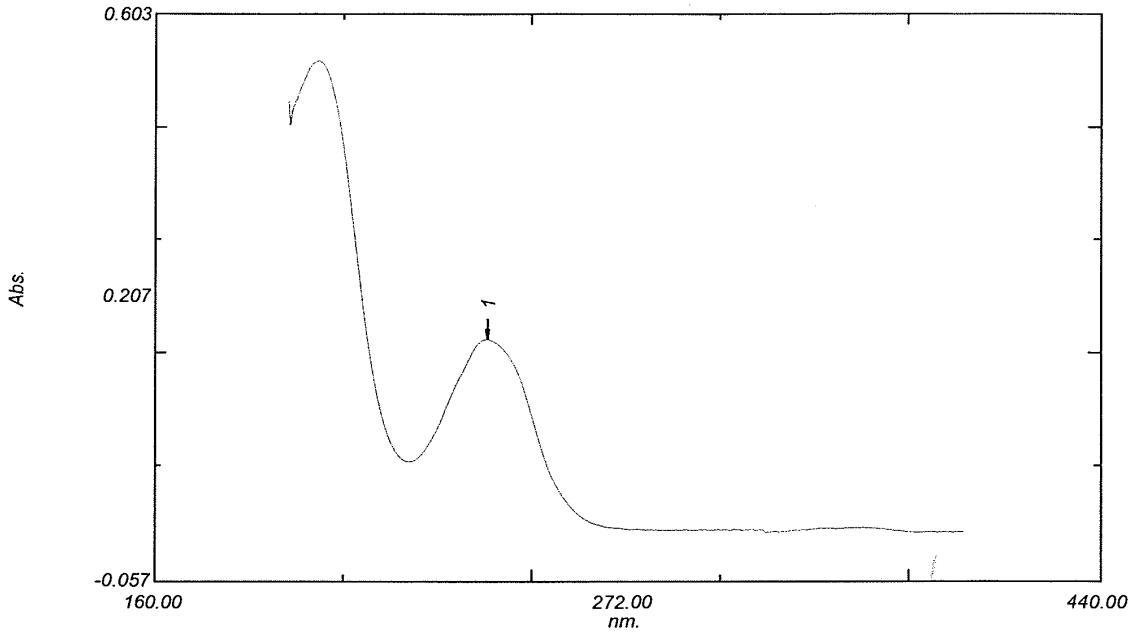
Sr.no	**Test**	**Specification**	**Result**
1	Loss on Drying(at 105^0C)	Not more than 1.5%w/w	1.2% w/w
2	Assay:	Not less than 98.0% w/w and Not more than 101% w/w	99.7% w/w
3	Density iii) Bulk iv) Tapped		 0.472 g/ml 0.810 g/ml
4	Compressibility Index		41.78%
5	Hausner Ratio		1.716
6	Angle of Repose		26
7	Particle Size #	Not less than 98.0% should pass through #22 mesh	100 % passed through #22 mesh

Result: From above result of tests performed the all values are within the limits, it was concluded that the received sample of Lamivudine was pure and might be used in the formulation

11.1.3 Identification of Drug

Determination of λ_{max} of Tenofovir DF:

On the basis of preliminary identification test it was concluded that the drug complied the preliminary identification. From the scanning of drug, it was concluded that the drug had λ_{max} of 259 nm, which was equal to 259 nm as reported. Also, an IR spectrum was concordant with the reference spectrum of Tenofovir DF.



Measurement Properties	
Wavelength Range (nm.):	200.00 to 400.00
Scan Speed:	Fast
Sampling Interval:	0.1
Auto Sampling Interval:	Disabled
Scan Mode:	Auto

No.	P/V	Wavelength	Abs.	Description
1	↑	259.00	0.222	

Fig. 11.1.3.1 UV Spectra of Tenofovir DF

Determination of λ_{max} of Lamivudine:

On the basis of preliminary identification test it was concluded that the drug complied the preliminary identification. From the scanning of drug, it was concluded that the drug had λ_{max} of 270 nm, which was equal to 270 nm as reported. Also, an IR spectrum was concordant with the reference spectrum of Lamivudine.

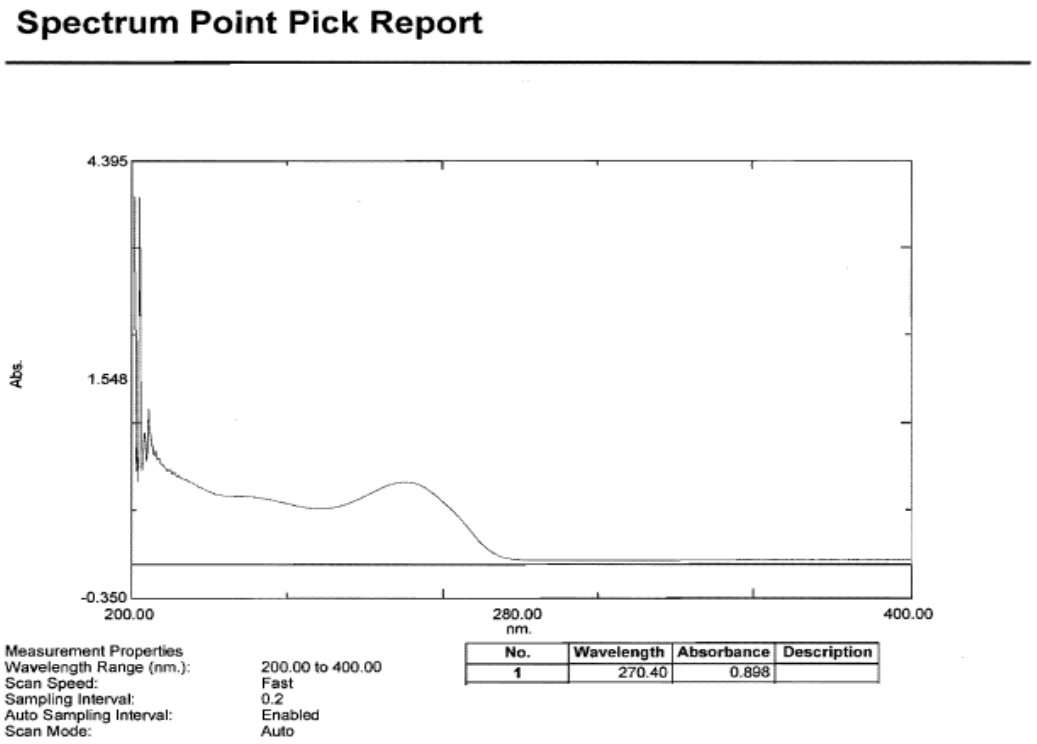


Fig. 11.1.3.2 UV Spectra of Lamivudine

Determination of IR spectrum of Tenofovir DF:

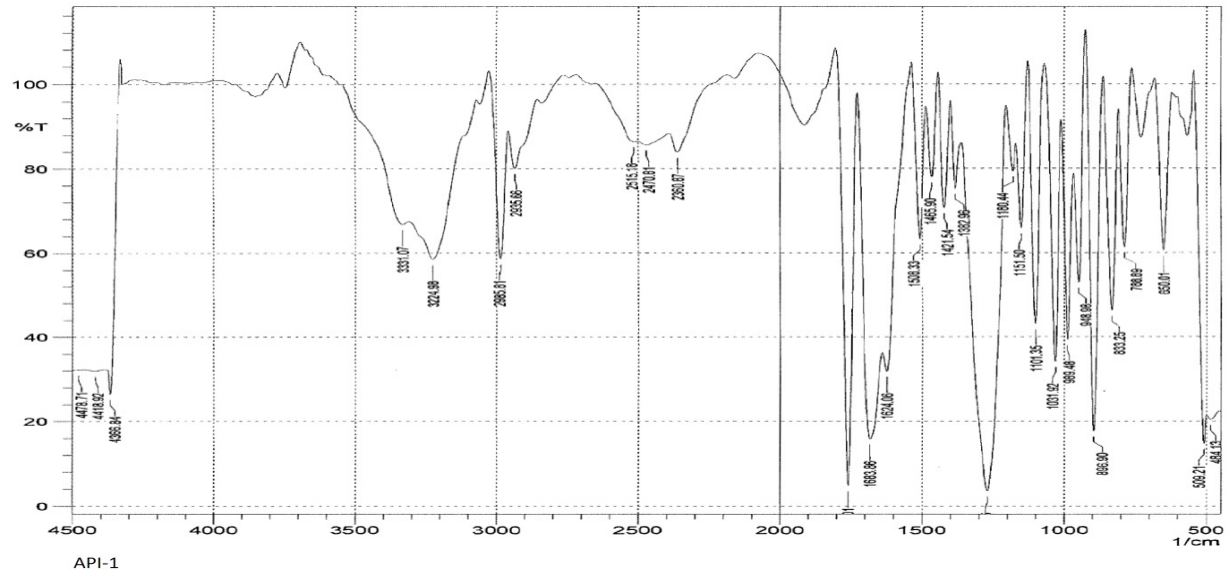


Fig. 11.1.3.3. IR SPECTRA OF TENOFOVIR DF

From the scanning of drug, it was concluded that the drug had, an IR spectrum that was concordant with the reference spectrum of Tenofovir DF

- **Solubility:** Tenofovir DF is freely soluble in water

Determination of IR spectrum of Lamivudine

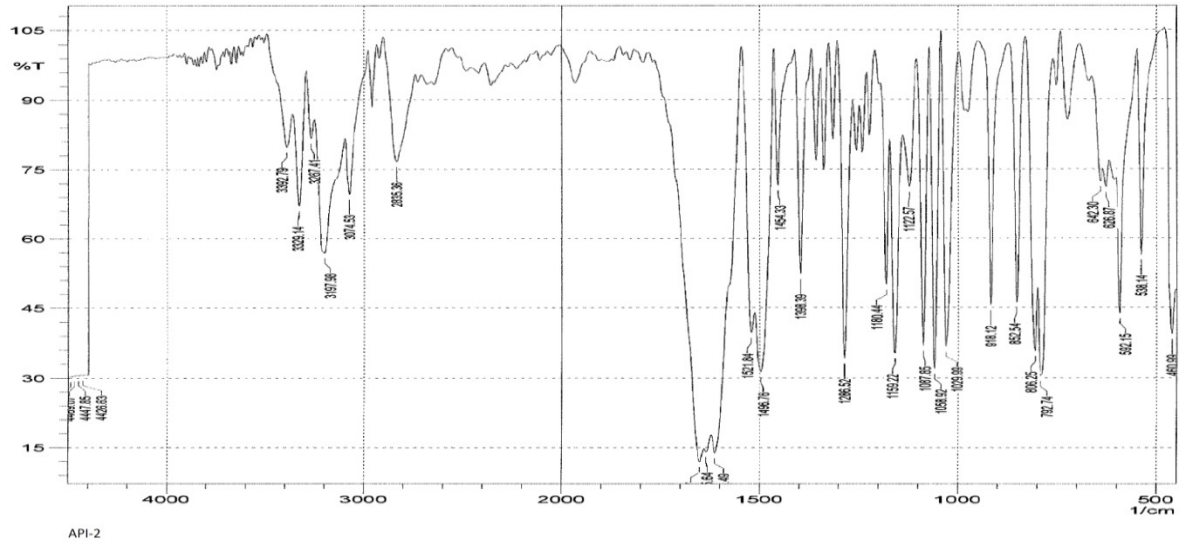


Fig. 11.1.3.4. IR SPECTRA OF LAMIVUDINE

From the scanning of drug, it was concluded that the drug had, an IR spectrum that was concordant with the reference spectrum of Lamivudine.

- Solubility: Lamivudine is freely soluble in water.

Determination of IR spectrum of Tenofovir DF +Lamivudine:

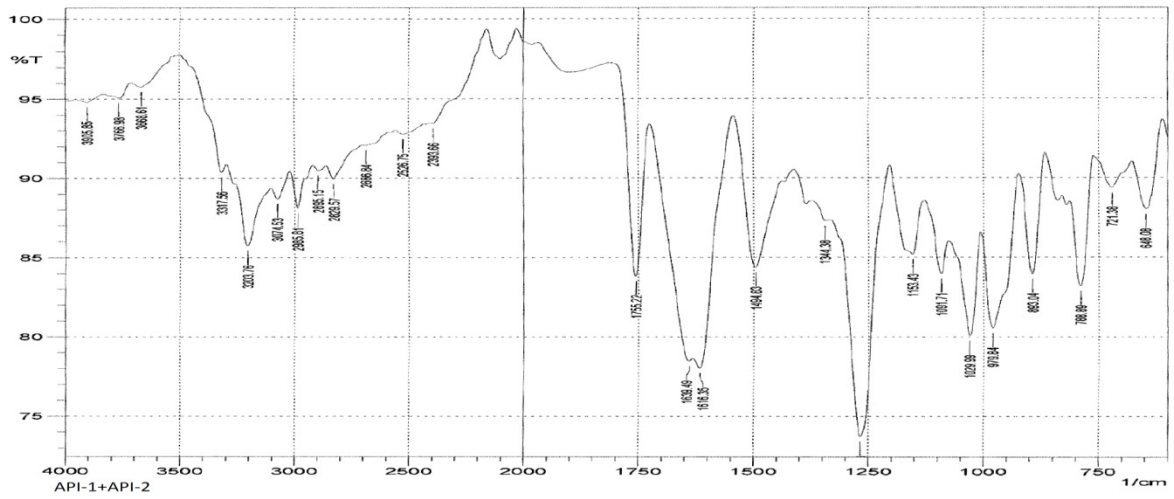


Fig.11.1.3.5. IR SPECTRA OF TENOFOVIR DF +LAMIVUDINE

11.2 DRUG EXCIPIENTS COMPATABILITY STUDIES

Results of drug excipient compatibility studies:

Drug-Excipient compatibility studies form an important part of preformulation studies for the determination of interaction between drug and excipient. It is determined after storage of specific time period by using suitable analytical techniques and the results are indicating that there is no interaction between drug and excipients.

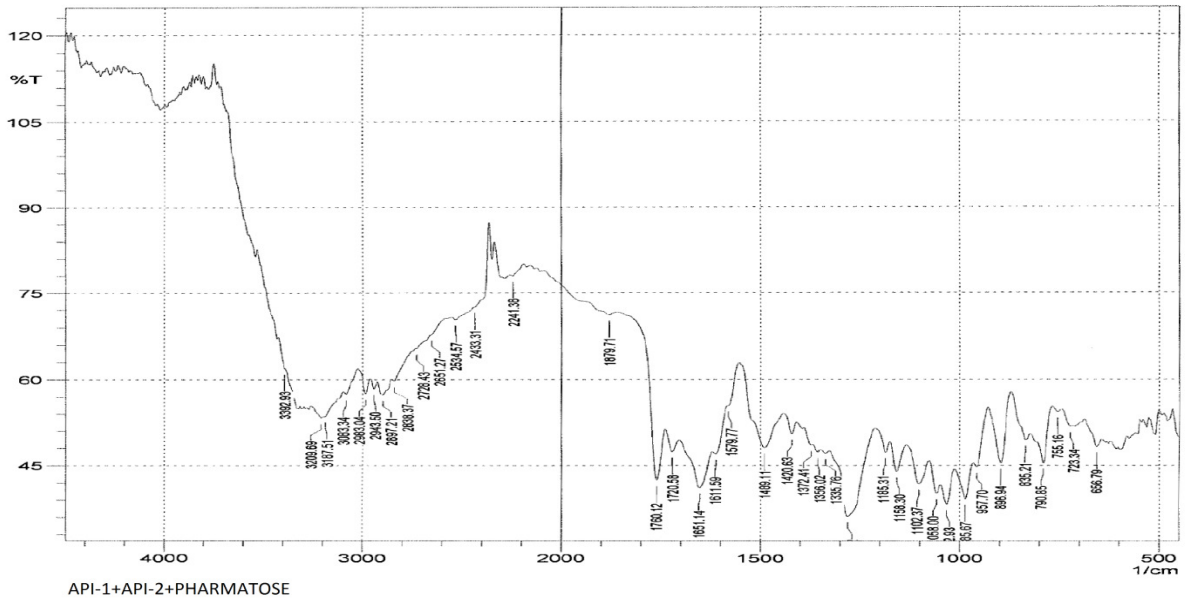


Fig. 11.2.I IR SPECTRA OF TENOFOVIR DF +LAMIVUDINE + LACTOSE MONOHYDRATE (PHARMATOSE 200M)

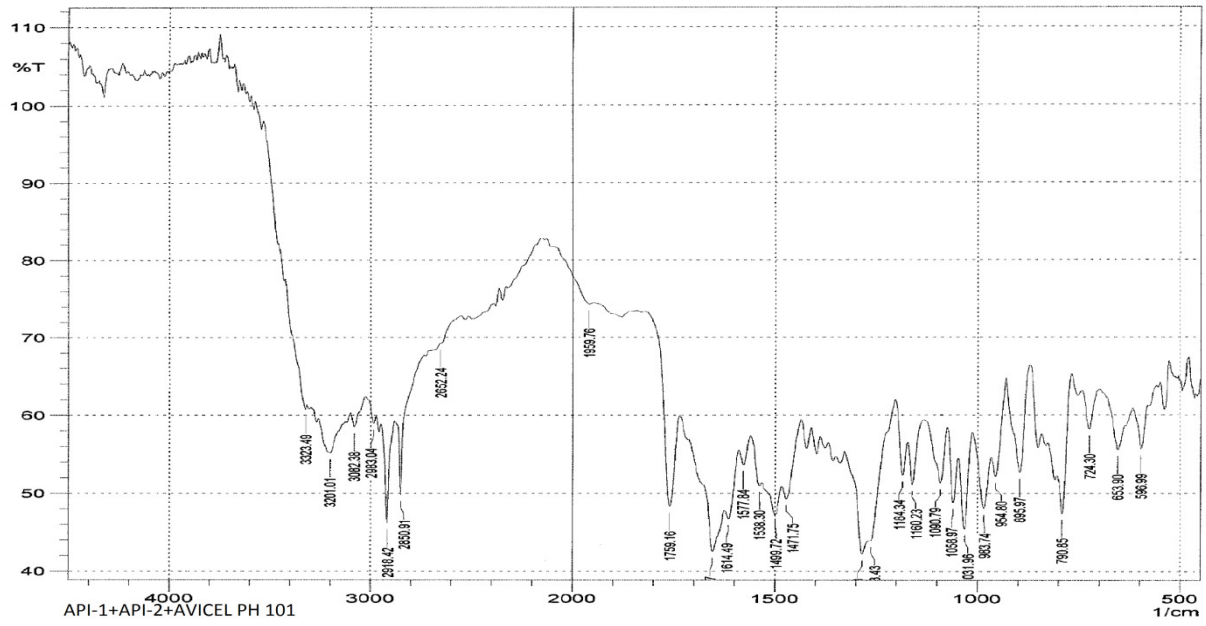


Fig.11.2.II IR SPECTRA OF TENOFOVIR DF +LAMIVUDINE +MCC (AVICEL PH 101)

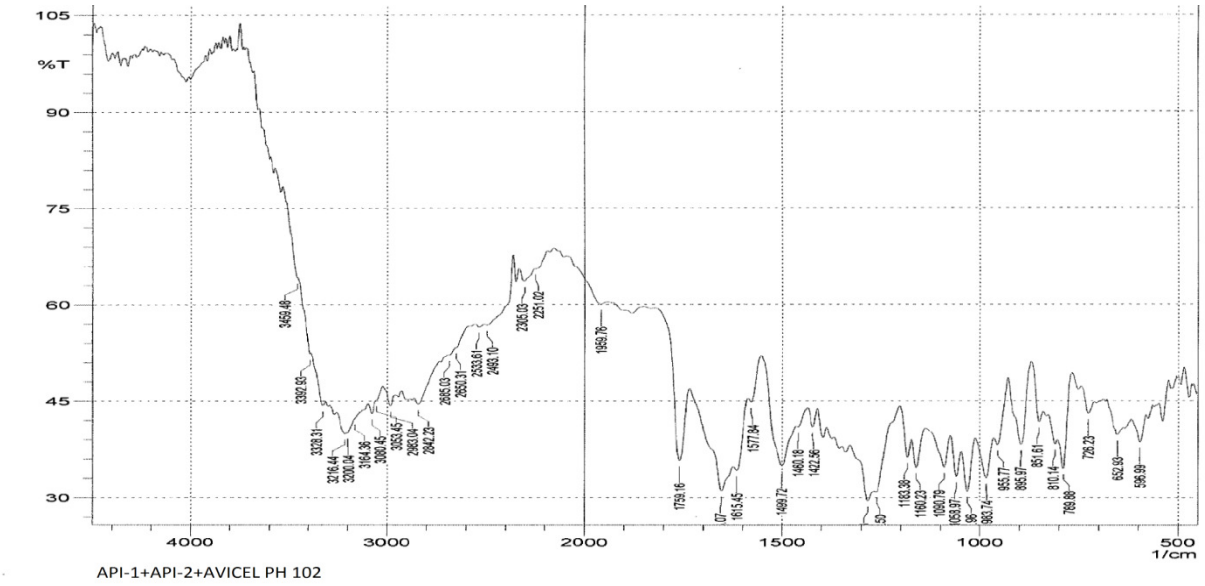


Fig.11.2.III IR SPECTRA OF TENOFOVIR DF +LAMIVUDINE +MCC (AVICEL PH 102)

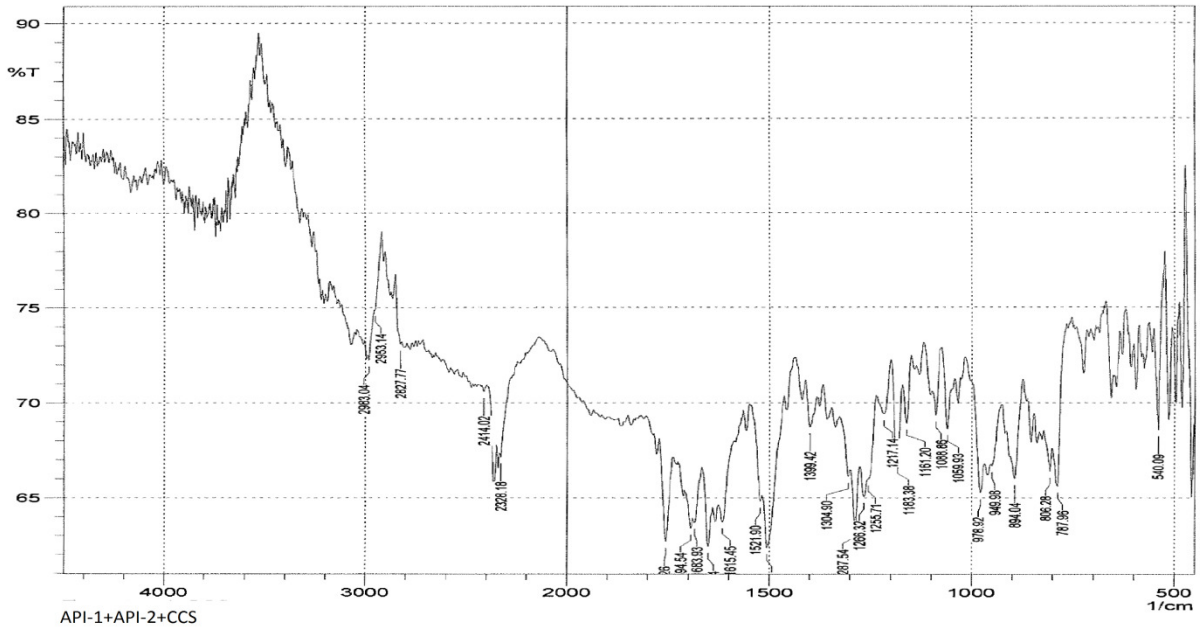


Fig. 11.2.IV IR SPECTRA OF TENOFOVIR DF +LAMIVUDINE +CCS

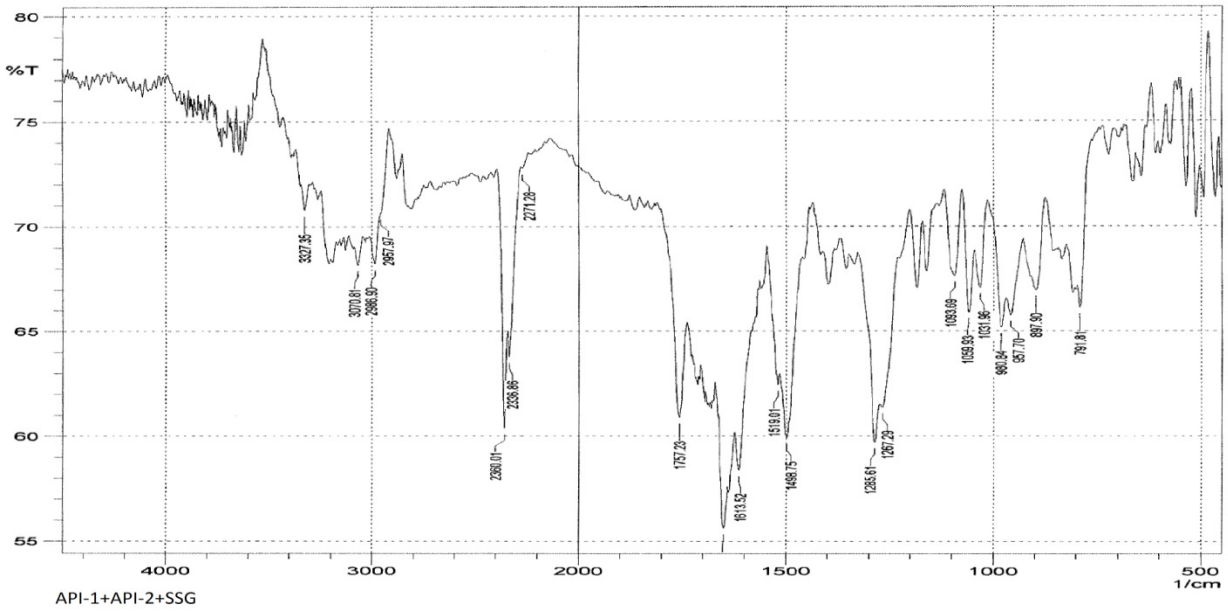


Fig.11.2.V IR SPECTRA OF TENOFOVIR DF +LAMIVUDINE +SSG

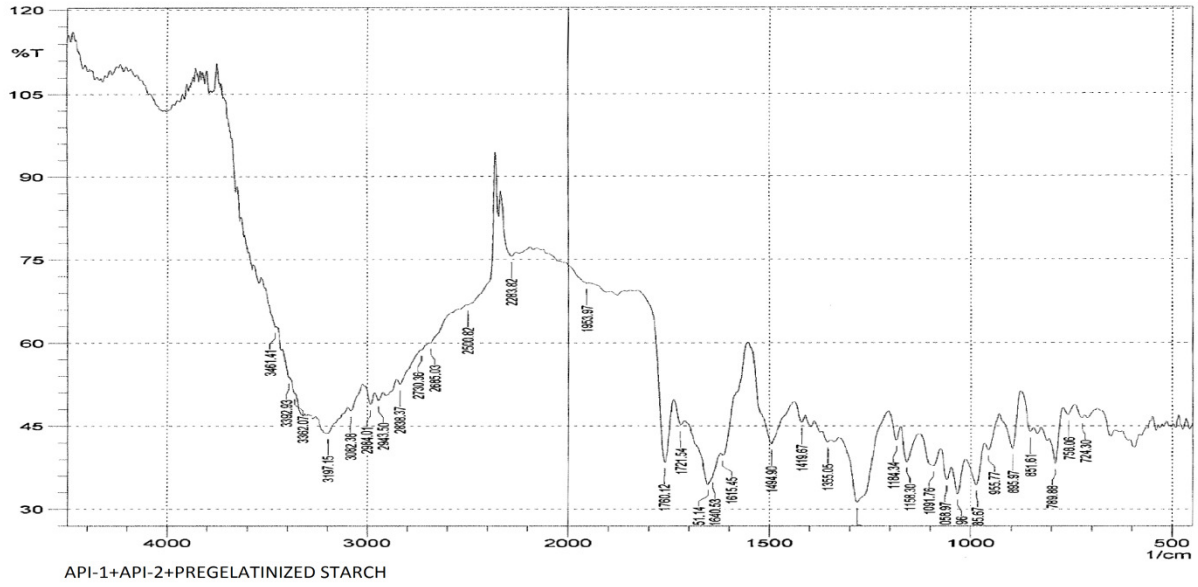


Fig. 11.2.VI IR SPECTRA OF TENOFOVIR DF +LAMIVUDINE + PREGELATINIZED STARCH (STARCH 1500)

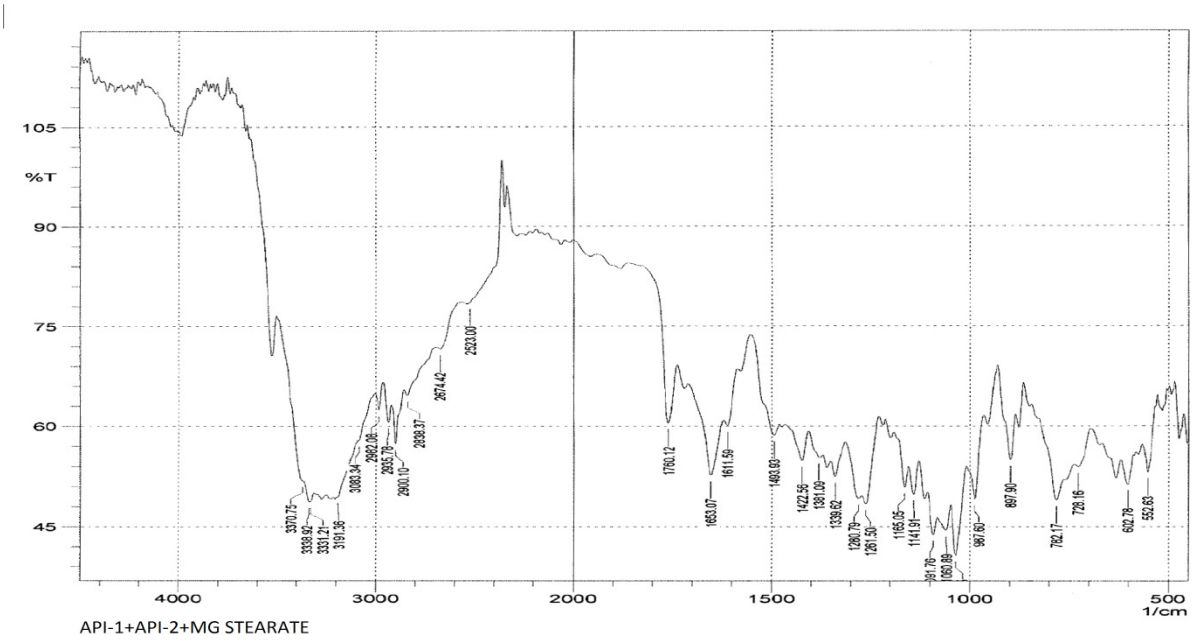


Fig.11.2.VII IR SPECTRA OF TENOFOVIR DF +LAMIVUDINE +MAGNESIUM STEARATE

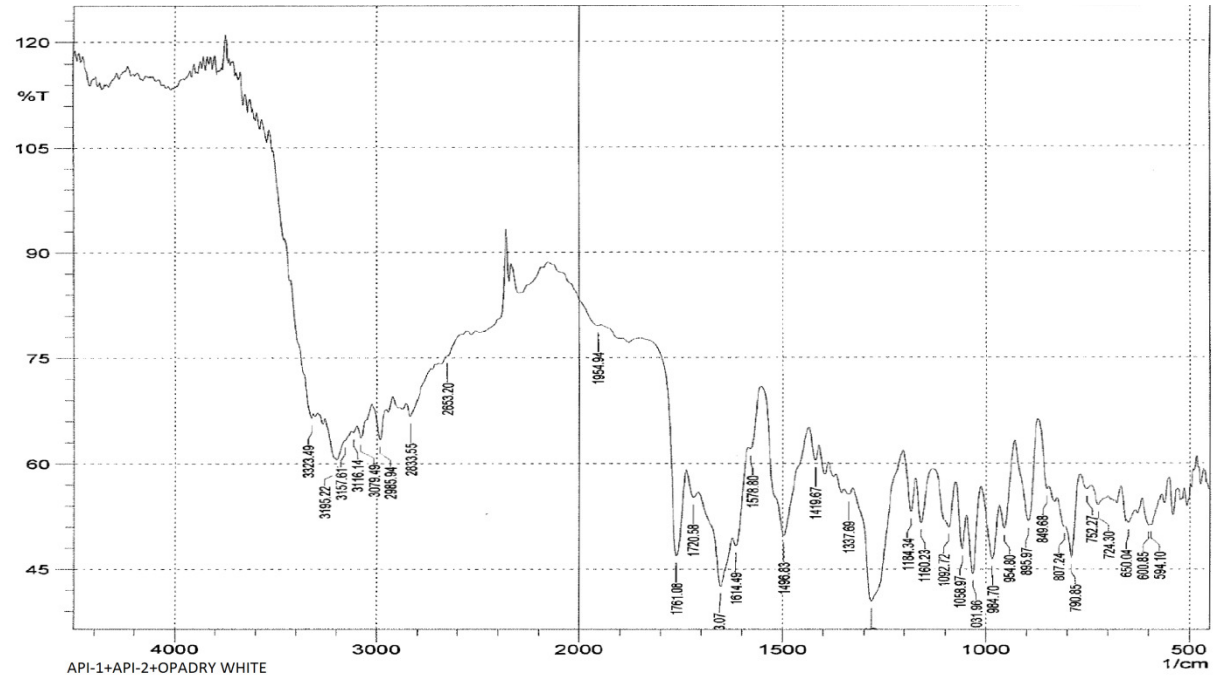


Fig. 11.2.VIII IR SPECTRA OF TENOFOVIR DF +LAMIVUDINE +OPADRY WHITE

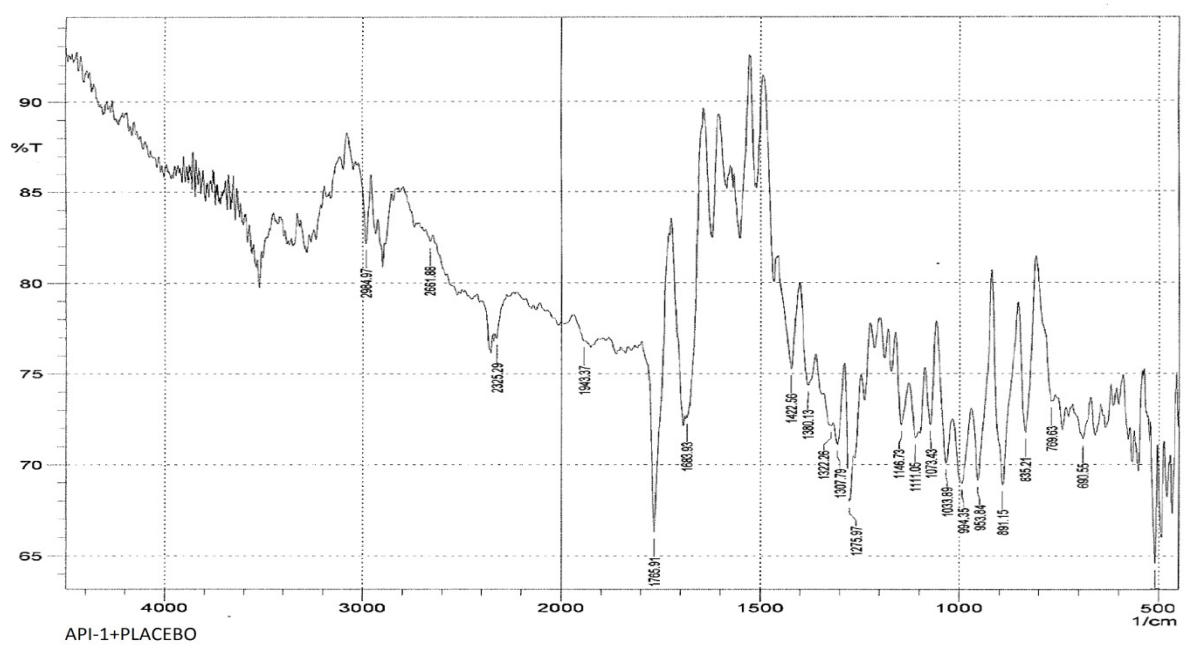


Fig.11.2.IX IR SPECTRA OF TENOFOVIR DF+ PLACEBO

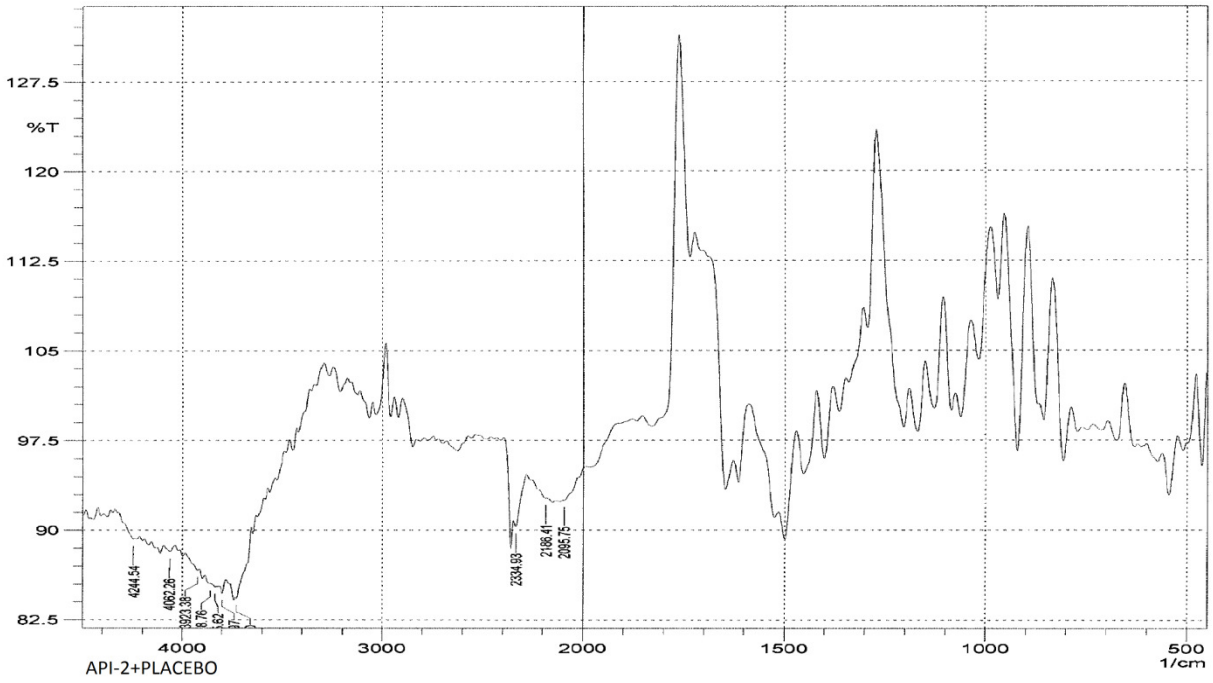


Fig. 11.2.X IR SPECTRA OF LAMIVUDINE + PLACEBO

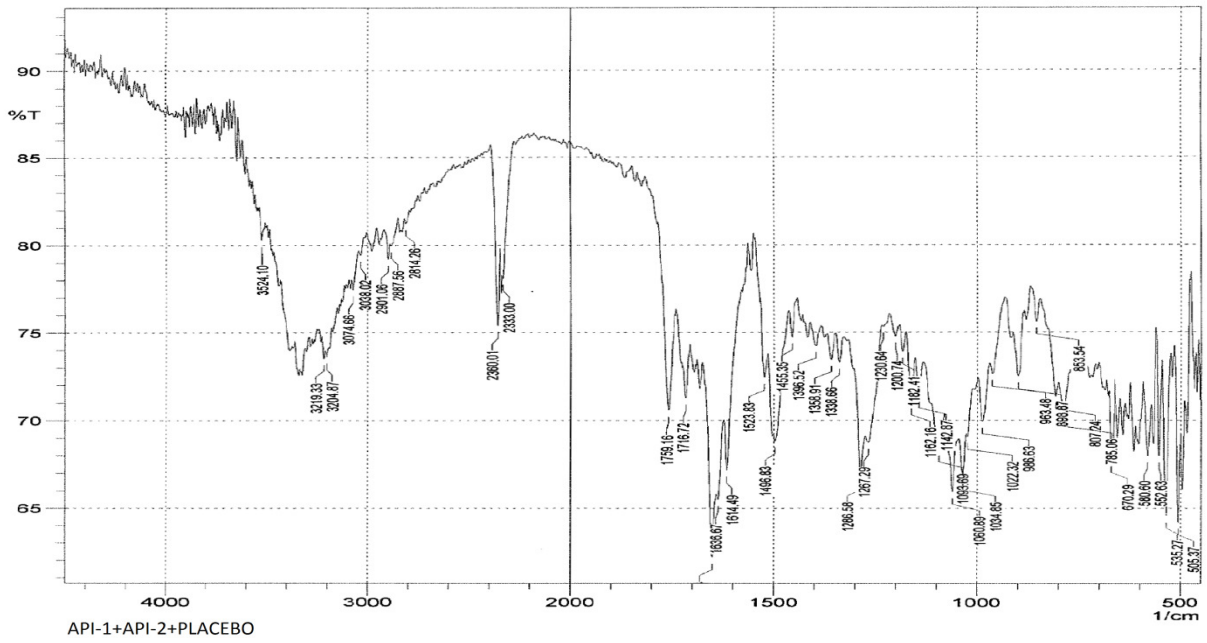


Fig.11.2.XI IR SPECTRA OF TENOFOVIR DF +LAMIVUDINE + PLACEBO

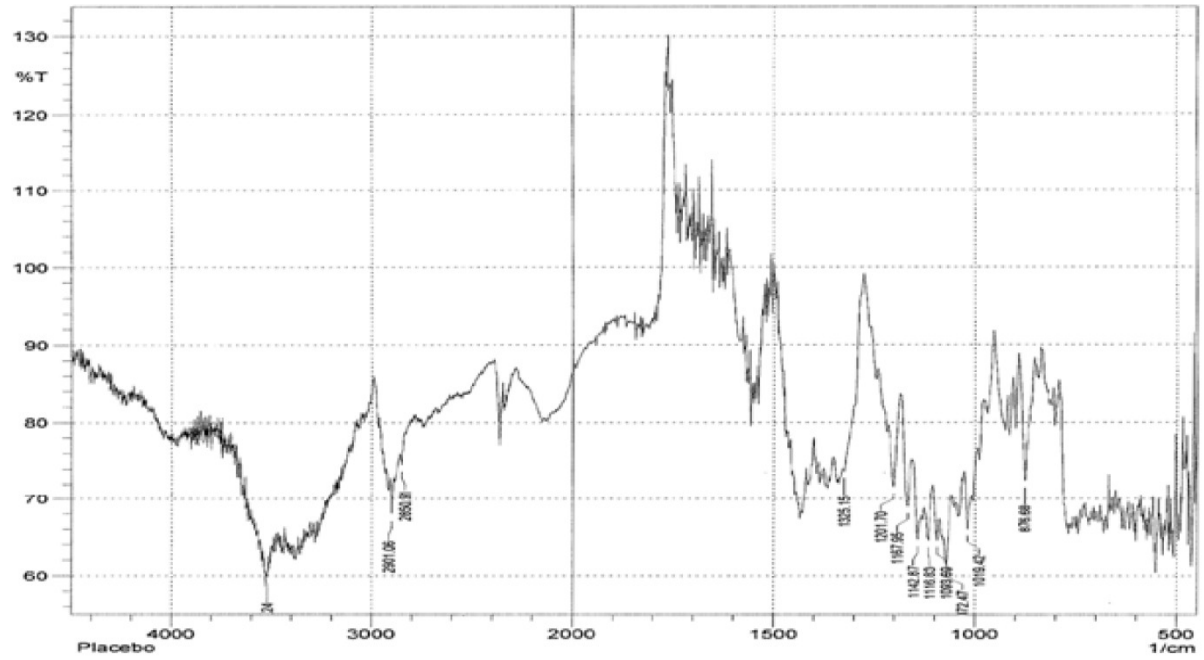


Fig.11.2.XII IR SPECTRA OF PLACEBO

TABLE NO.11.2.1 Drug-exicipient compatibility Result:

Sr. No	Name of the Excipient	Ratio API: Expt	Initial Observation	Final observation		Conclusion
				40°C/75% RH		
				2nd week	4th week	
1	Tenofovir DF	-	White to off white	White to off white	White to off white	Compatible
2	Lamivudine		White to off white	White to off white	White to off white	Compatible
3	Tenofovir DF +Lamivudine	-	White to off white	White to off white	White to off white	Compatible
4	Tenofovir DF +Lactose monohydrate(Pharmatose 200m)	1:2	White fine powder	White fine powder	White fine powder	Compatible
5	Tenofovir DF + Microcrystalline Cellulose (Avicel PH101)	1:2	off-white	off-white	off-white	Compatible
6	Tenofovir DF + Microcrystalline Cellulose (Avicel PH101)	1:2	off-white	off-white	off-white	Compatible
7	Tenofovir DF + Pregelatinized Starch (Starch 1500)	1:0.5	off-white	off-white	off-white	Compatible
8	Tenofovir DF+ Sodium starch glycolate-A	1:0.5	White	White	White	Compatible
9	Tenofovir DF+ Croscarmellose sodium(Primellose)	1:0.5	Off white	Off white	Off white	Compatible
10	Tenofovir DF+ Magnesium stearate	1:0.05	White	White	White	Compatible
11	Tenofovir DF+ Opadry White	1:0.25	White	White	White	Compatible

12	Lamivudine +Lactose monohydrate(Pharmatose 200m)	1:2	White fine powder	White fine powder	White fine powder	Compatible
13	Lamivudine + Microcrystalline Cellulose (Avicel PH101)	1:2	off-white	off-white	off-white	Compatible
14	Lamivudine + Microcrystalline Cellulose (Avicel PH101)	1:2	off-white	off-white	off-white	Compatible
15	Lamivudine + Pregelatinized Starch (Starch 1500)	1:0.5	off-white	off-white	off-white	Compatible
16	Lamivudine+ Sodium starch glycolate-A	1:0.5	White	White	White	Compatible
17	Lamivudine+ Croscarmellose sodium(Primellose)	1:0.5	Off white	Off white	Off white	Compatible
18	Lamivudine+ Magnesium stearate	1:0.05	White	White	White	Compatible
19	Lamivudine+ Opadry White	1:0.25	White	White	White	Compatible
20	Tenofovir DF + Lamivudine+Lactose monohydrate(Pharmatose 200m)	1:2	White fine powder	White fine powder	White fine powder	Compatible
21	Tenofovir DF + Lamivudine+ Microcrystalline Cellulose (Avicel PH101)	1:2	off-white	off-white	off-white	Compatible
22	Tenofovir DF +Lamivudine + Microcrystalline Cellulose (Avicel PH101)	1:2	off-white	off-white	off-white	Compatible
23	Tenofovir DF + Lamivudine+ Pregelatinized Starch (Starch 1500)	1:0.5	off-white	off-white	off-white	Compatible

24	Tenofovir DF + Lamivudine+ Sodium starch glycolate-A	1:0.5	White	White	White	Compatible
25	Tenofovir DF + Lamivudine+ Croscarmellose sodium(Primellose)	1:0.5	Off white	Off white	Off white	Compatible
26	Tenofovir DF + Lamivudine+ Magnesium stearate	1:0.05	White	White	White	Compatible
27	Tenofovir DF + Lamivudine+ Opadry White	1:0.25	White	White	White	Compatible
28	Tenofovir DF+ Placebo	1:2	Off white	Off white	Off white	Compatible
29	Lamivudine+ Placebo	1:2	Off white	Off white	Off white	Compatible
30	Tenofovir DF + Lamivudine+ Placebo	1:2	Off white	Off white	Off white	Compatible

It was concluded that there was no interference in the functional group as the principle peaks of the Tenofovir DF and Lamivudine were found to be unaltered in the drug-polymer physical mixture, indicating they were compatible chemically.

11.3 PREPARATION OF STANDARD CURVE

11.3.1 PREPARATION OF STANDARD CURVE OF TENOFOVIR DF:

From the standard curve of Tenofovir DF was observed that the drug obeys beer's law in concentration range of 2-10 µg/ml in0.1N HCl. The linear regression equation generated was used for the calculation of amount of drug.

Table.11.3.1 Standard curve of Tenofovir DF in 0.1N HCl

Sr. No.	Conc.(µg/ml)	Absorbance
1	2	0.051
2	4	0.099
3	6	0.149
4	8	0.202
5	10	0.239

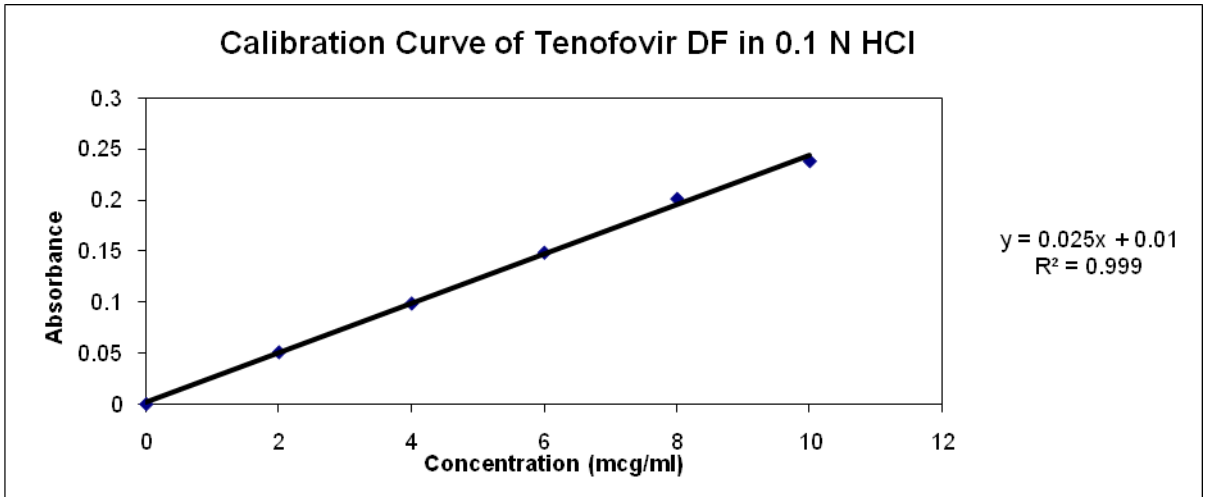


Fig. 11.3.1 Standard curve of Tenofovir DFin 0.1N HCl

11.3.2 PREPARATION OF STANDARD CURVE OF LAMIVUDINE:

From the standard curve of Lamivudine was observed that the drug obeys beer's law in concentration range of 2-10 µg/ml in0.1N HCl. The linear regression equation generated was used for the calculation of amount of drug.

Table.11.3.2 Standard curve of Lamivudine in 0.1N HCl

Sr. No.	Conc.(µg/ml)	Absorbance
1	2	0.121
2	4	0.223
3	6	0.315
4	8	0.421
5	10	0.515

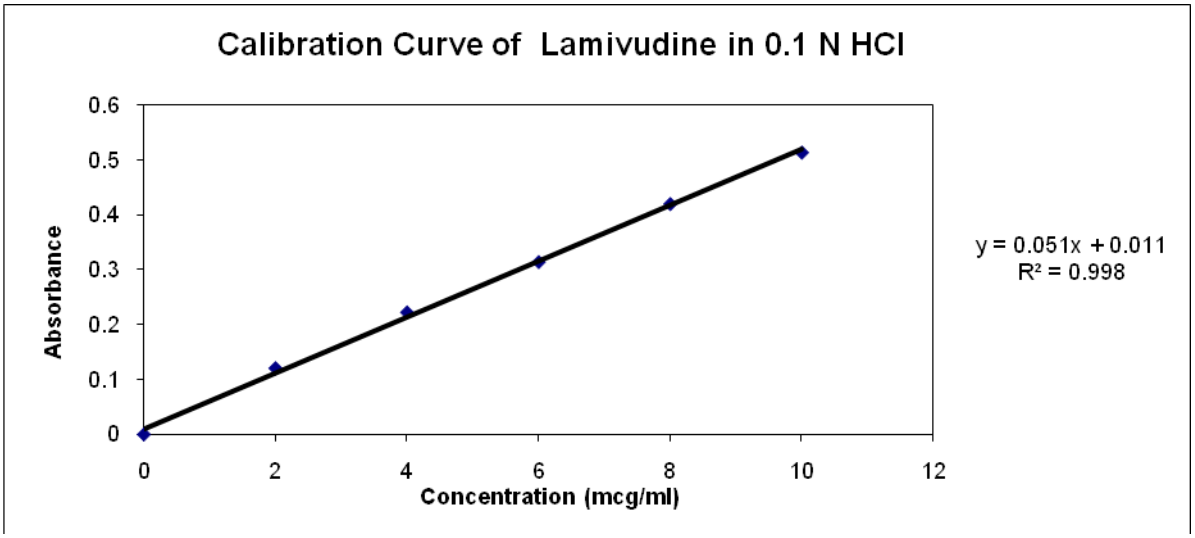


Fig. 11.3.2 Standard curve of Lamivudine in 0.1N HCl

11.4 PREFORMULATION STUDIES OF GRANULES:

Table No. 11.4.1 Flow properties of blends of different formulations

BATCH	BULK DENSITY (gm/ml)	TAPPED DENSITY (gm/ml)	CARR'S INDEX (%)	HAUSNER'S RATIO	RESULT
Tenofovir DF	0.412	0.636	35.29	1.54	Very poor
Lamivudine	0.472	0.810	41.66	1.71	Extremly poor
F1	0.601	0.860	30.1	1.43	Poor
F2	0.645	0.833	22.58	1.29	Poor
F3	0.560	0.800	30.5	1.46	Poor
F4	0.550	0.790	30.37	1.43	Poor
F5	0.450	0.590	23.70	1.27	Poor
F6	0.420	0.550	23.63	1.30	Poor
F7	_	_	_	_	_
F8	_	_	_	_	_
F9	_	_	_	_	_
F10	_	_	_	_	_
F11	_	_	_	_	_
F12	_	_	_	_	_

Table No. 11.4.2 Flow properties of Tenofovir DF blends of different formulations

BATCH	BULK DENSITY (gm/ml)	TAPPED DENSITY (gm/ml)	CARR'S INDEX (%)	HAUSNER'S RATIO	RESULT
F1	_	_	_	_	_
F2	_	_	_	_	_
F3	0.650	0.870	25.86	1.33	Poor
F4	0.650	0852	25.46	1.30	Poor
F5	0.465	0.598	22.24	1.28	Poor
F6	0.454	0.580	21.72	1.27	Poor

F7	0.43	0.56	23.21	1.3	Poor
F8	0.445	0.56	20.54	1.25	Fair
F9	0.44	0.55	20.00	1.25	Fair
F10	0.45	0.55	18.18	1.22	Fair
F11	0.44	0.56	21.43	1.27	Fair
F12	0.45	0.56	19.64	1.24	Fair

Table No. 11.4.3 Flow properties of Lamivudine blends of different formulations

BATCH	BULK DENSITY (gm/ml)	TAPPED DENSITY (gm/ml)	CARR'S INDEX (%)	HAUSNER'S RATIO	RESULT
F1	_	_	_	_	_
F2	_	_	_	_	_
F3	0.526	0.833	36.85	1.58	poor
F4	0.530	0.833	36.85	1.58	poor
F5	0.447	0.572	21.85	1.27	Fair
F6	0.435	0.571	23.81	1.31	poor
F7	0.432	0.532	18.8	1.23	Fair
F8	0.430	0.530	18.86	1.23	Fair
F9	0.435	0.54	19.44	1.24	Fair
F10	0.430	0.540	20.37	1.25	Fair
F11	0.425	0.535	20.56	1.25	Fair
F12	0.470	0.58	18.96	1.23	Fair

Result: Preformulation study of powder blend had shown that the blend had passable parameters like Angle of Repose, Bulk density, Tapped density, Carr's index and Hausner's ratio.

11.5 EVALUATION PARAMETERS OF TABLETS

Table no: 11.5.1 Evaluation parameters of formulations for uncoated tablets

Formulation code	Evaluation parameter						
	Thickness ± S.D. (mm) (n = 5)	Hardness ± S.D. (KP) (n = 5)	Friability (%)	Average weight variation (n=20)	Drug content (Tenofovir DF) (%)	Drug content (Lamivudine) (%)	Disintegration Time
F1	6.40±0.10	11.00±1.0	0.74	760±5.00	97.18	97.13	10-12 min
F2	6.00±0.10	12.00±1.0	0.9	760±5.00	96.98	97.27	10-12min
F3	6.40±0.10	12.00±1.0	0.35	900±8.00	98.36	97.03	10-12 min
F4	6.40±0.10	10.00±1.0	0.41	900±6.00	97.65	98.63	7-9 min
F5	6.60±0.10	16.00±1.5	0.42	1000±5.00	97.59	98.33	3-4 min
F6	7.10±0.10	15.00±1.0	0.31	1220±5.00	98.41	98.46	3-4 min
F7	7.10±0.10	15.00±1.0	0.29	1225±5.00	98.52	98.76	2-3 min

F8	7.10±0.10	15.00±1.0	0.25	1225±5.00	98.21	98.78	1-2 min
F9	7.10±0.10	15.00±1.0	0.31	1225±5.00	98.90	98.47	1-2 min
F10	7.00±0.10	15.00±1.0	0.28	1225±5.00	98.66	98.36	2-3 min
F11	7.10±0.10	17.00±1.0	0.26	1225±5.00	98.56	98.07	2-3 min
F12	7.00±0.10	17.00±1.5	0.24	1225±5.00	97.36	98.45	2-3 min

Tableno.11.5.2 Evaluation parameters of formulations for film coated tablets

11.6 PHYSICOCHEMICAL EVALUATION:

Formulation code	Evaluation parameter			
	Thickness ± S.D. (mm) (n = 5)	**Hardness ± S.D. (Kp) (n = 5)**	**Average weight variation (n=20)**	**Disintegration Time**
F1	6.50±0.10	17.0±1.0	780±6.00	10-12 min
F2	6.50±0.10	17.0±1.0	780±6.00	10-12 min
F3	6.50±0.10	15.0±2.0	930±8.00	10-12 min
F4	6.40±0.10	15.0±2.0	930±8.00	8-10 min
F5	6.80±0.10	22.0±2.0	1030±5.00	4-5 min
F6	7.40±0.10	15.0±1.0	1260±5.00	3-5 min
F7	7.40±0.10	25.0±1.0	1265±8.00	2-3 min
F8	7.40±0.10	25.0±1.0	1265±8.00	2-3 min
F9	7.40±0.10	25.0±1.0	1265±8.00	2-3 min
F10	7.25±0.10	22.0±2.0	1265±5.00	2-3 min
F11	7.40±0.10	25.0±2.0	1265±8.00	3-4 min
F12	7.30±0.10	22.0±2.0	1265±5.00	2-3 min

The prepared tablets were subjected to preliminary characterization such as hardness, thickness, % weight variation, friability and drug content. The evaluated parameters were within acceptable range for all the formulations. The values are indicated in below Table.

Table no: 11.6.1 Range for value of preliminary characterization of formulations

Parameters	**Range**
Hardness (kp)	15.0-25.0
Thickness (mm)	6.50-7.40
Weight variation (%)	±5%
% Friability	Not more than 1.0%
Assay(%)	96.98-98.90

Result: Evaluation parameters of formulation, it showed that the all the parameters viz. hardness, thickness, friability, average weight and content uniformity were in the passable range.

11.7 RESULTS OF IN-VITRO RELEASE PROFILE

11.7.1 Results of In-Vitro Release Profile of Tenofovir DF:

Table.11.7.1.1 In-Vitro Release Profile

Sr. No.	Time (min)	F1	F2	F3	F4	F5	F6
1	5	10.08	15.36	16.32	26.88	36.48	37.44
2	10	18.24	21.12	22.08	34.08	47.04	48.48
3	15	26.88	29.28	31.2	44.64	57.12	56.64
4	20	35.04	37.44	38.4	56.16	65.28	67.68
5	30	42.24	44.16	45.12	63.36	72.96	73.92
6	45	52.32	52.8	52.32	69.6	79.68	83.52
7	60	65.28	66.72	66.24	77.28	85.92	93.12

Table.11.7.1.2 In-Vitro Release Profile

Sr. No.	Time (min)	F7	F8	F9	F10	F11	F12
1	5	61.92	72.48	72.48	72.96	71.04	68.64
2	10	72.96	80.16	85.92	85.44	80.16	79.2
3	15	82.08	85.92	88.8	88.32	85.92	86.4
4	20	85.92	93.12	93.12	94.08	89.28	90.24
5	30	89.76	95.04	95.04	96.48	91.68	93.12
6	45	94.08	98.88	99.84	99.84	99.36	99.36

7	60	101.76	102.24	104.64	102.24	103.2	104.64

11.7.2 Results of IN-VITRO release profile of Lamivudine:

Table.11.7.2.1 In-Vitro Release Profile

Sr. No.	Time (min)	F1	F2	F3	F4	F5	F6
1	5	22.35	24.70	28.47	29.64	64.94	65.64
2	10	29.41	31.76	37.17	38.11	74.35	73.41
3	15	38.11	42.58	45.88	46.58	82.11	82.82
4	20	46.58	52.70	55.05	55.52	91.05	90.35
5	30	55.29	63.29	67.29	67.29	96.70	93.17
6	45	64.70	72.47	77.88	79.05	100.70	99.05
7	60	75.52	85.17	87.52	88	103.76	101.4

Table.11.7.2.2 In-Vitro Release Profile

Sr. No.	Time(min)	F7	F8	F9	F10	F11	F12
1	5	82.823	85.17	89.17	87.29	87.05	86.58
2	10	86.11	89.17	92.23	90.35	91.29	92.70
3	15	92.23	90.35	94.35	94.82	95.29	96
4	20	94.35	95.29	96.23	96.23	97.17	97.41
5	30	96.23	97.88	97.64	99.05	98.823	98.11
6	45	97.88	100.23	100.23	100.23	100.23	100.79
7	60	102.11	103.05	104.23	102.11	102.11	103.29

Table No: 11.7.3.2 In-Vitro Release Profile of Innovator of Tenofovir DF:

Sr. No.	Time(min)	%DR
1	5	74.4
2	10	88.32
3	15	90.72
4	20	92.16
5	30	94.08
6	45	100.32
7	60	105.6

Table No: 11.7.3.2 In-Vitro Release Profile of Innovator of Lamivudine:

Sr. No.	Time(min)	%DR
1	5	88.94
2	10	91.76
3	15	94.58
4	20	96.47
5	30	97.41
6	45	100.23
7	60	103.52

Graphical presentation of Dissolution in0.1N HCl of Tenofovir DF:

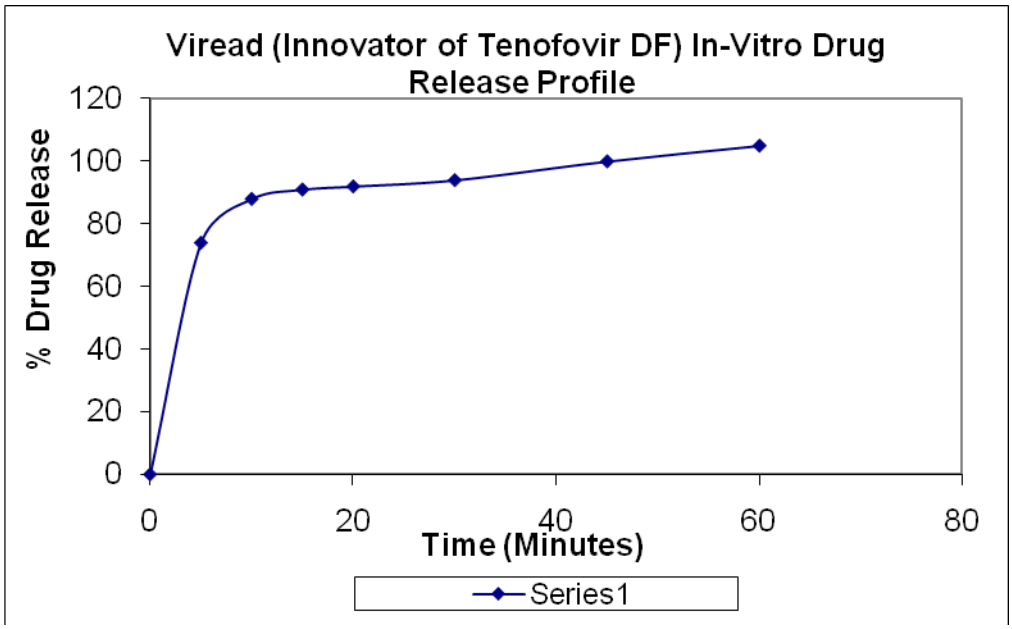


Fig. 11.7.I In-Vitro Release Profile of Viread (Innovator of Tenofovir DF)

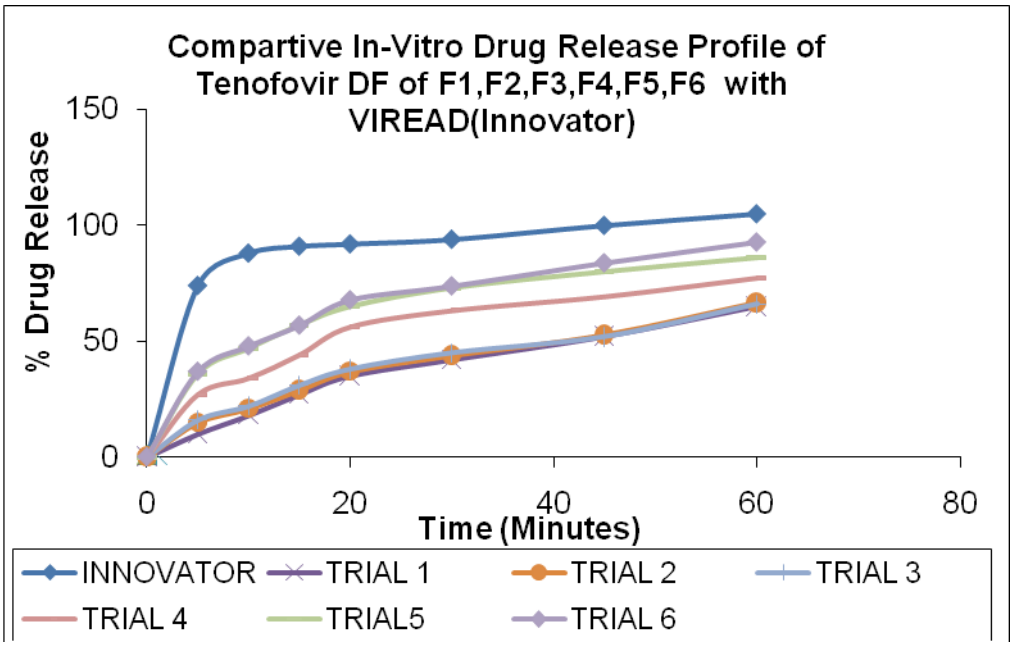


Fig.11.7.II In-Vitro Release Profile of F1,F2,F3,F4,F5,F6 with Innovator (Viread)

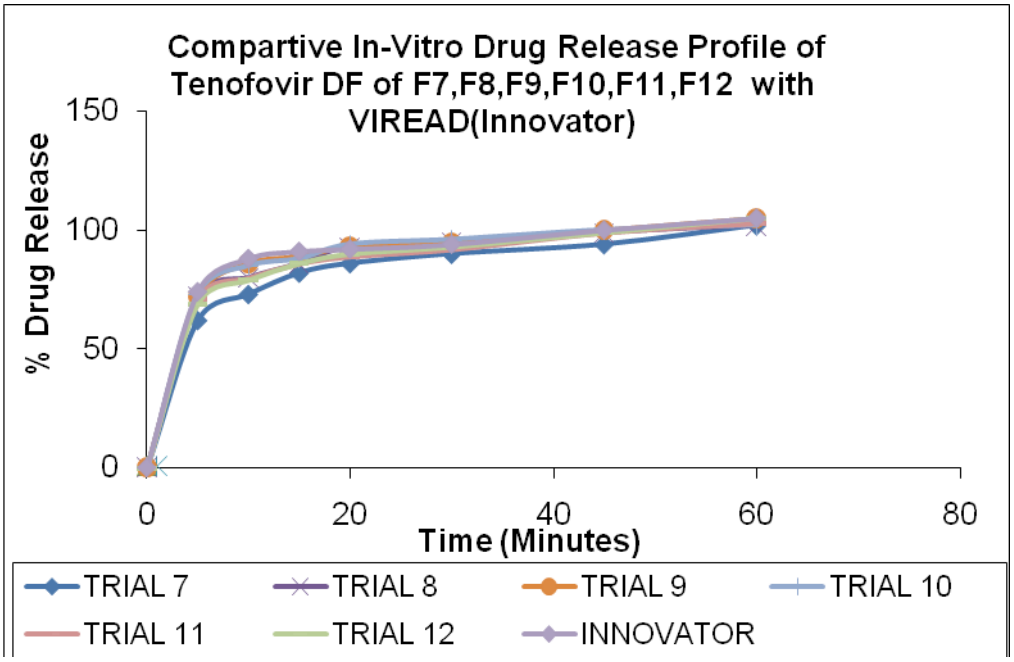


Fig.11.7.III In-Vitro Release Profile of F7,F8,F9,F10,F11,F12 with Innovator (Viread)

Graphical presentation of Dissolution in0.1N HCl of Lamivudine:

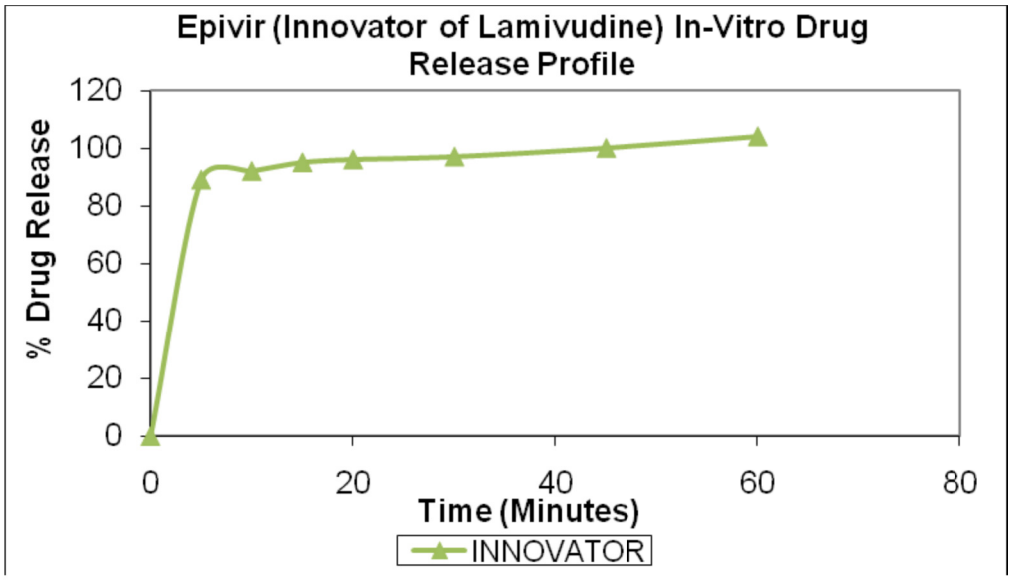


Fig. 11.7 IV In-Vitro Release Profile of Epivir (Innovator of Lamivudine)

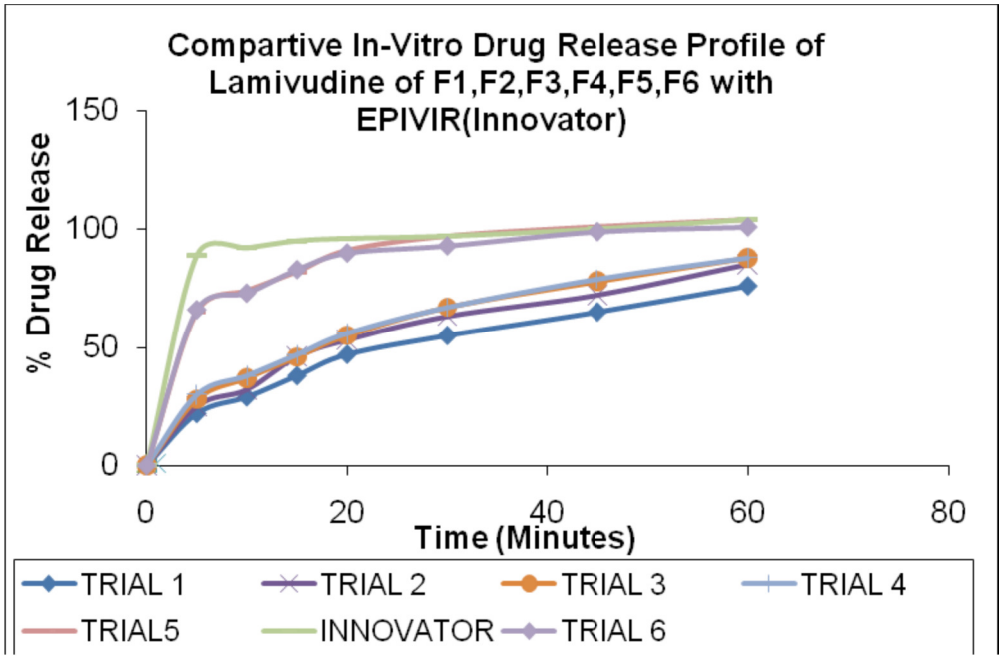


Fig.11.7.V In-Vitro Release Profile of F1,F2,F3,F4,F5,F6 with Innovator(Epivir)

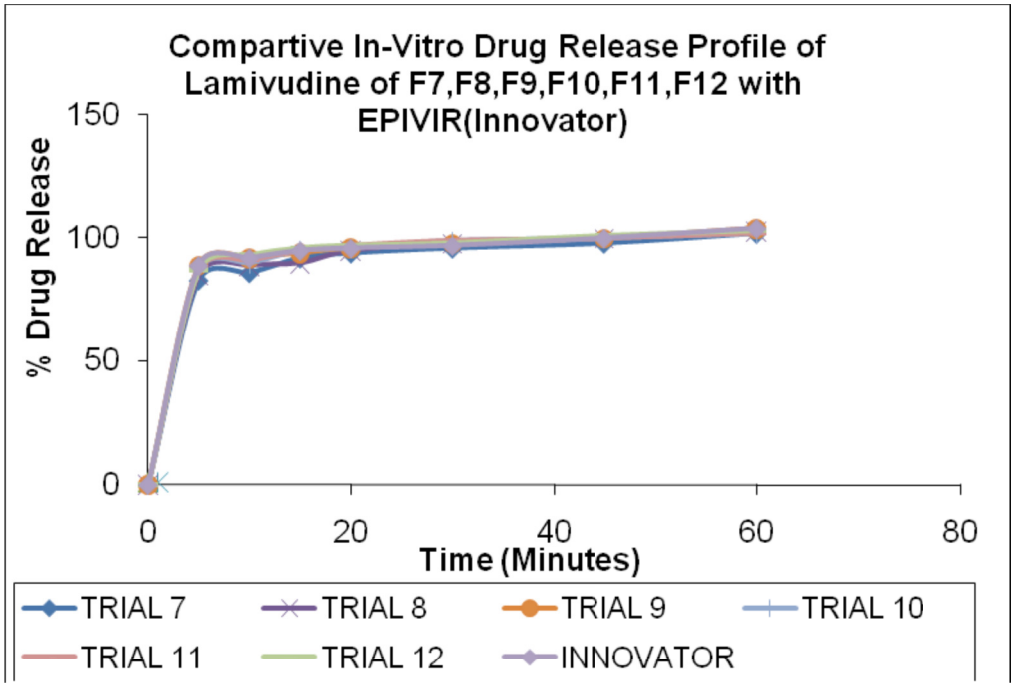


Fig. 11.7.VI I In-Vitro Release Profile of F7,F8,F9,F10,F11,F12 with Innovator

Comparative In-vitro release profile with innovator

Table no11.7.3.3 Comparative In-vitro release profile with innovator of Tenofovir DF

Sr. No.	Time(min)	Viread	F9
1	**5**	74.4	72.48
2	10	88.32	85.92
3	15	90.72	88.8
4	20	92.16	93.12
5	30	94.08	95.04
6	45	100.32	99.84
7	60	105.6	104.64

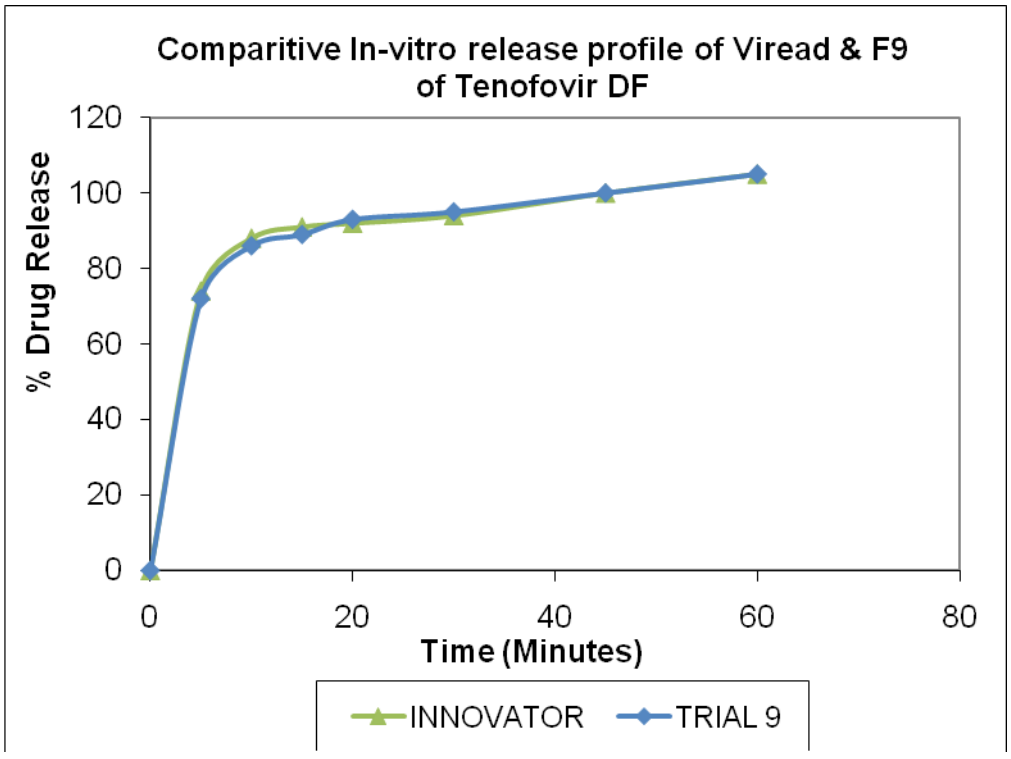


Fig.11.7.VII In-Vitro Release Profile of F9 with Viread (Innovator)

Table no11.7.3.4.Comparative In-vitro release profile with innovator of Lamivudine

Sr. No.	Time(min)	Epivir	F9
1	**5**	88.94	87.52

2	10	91.76	91.29
3	15	94.58	93.88
4	20	96.47	94.823
5	30	97.41	97.88
6	45	100.25	100.23
7	60	103.52	102.11

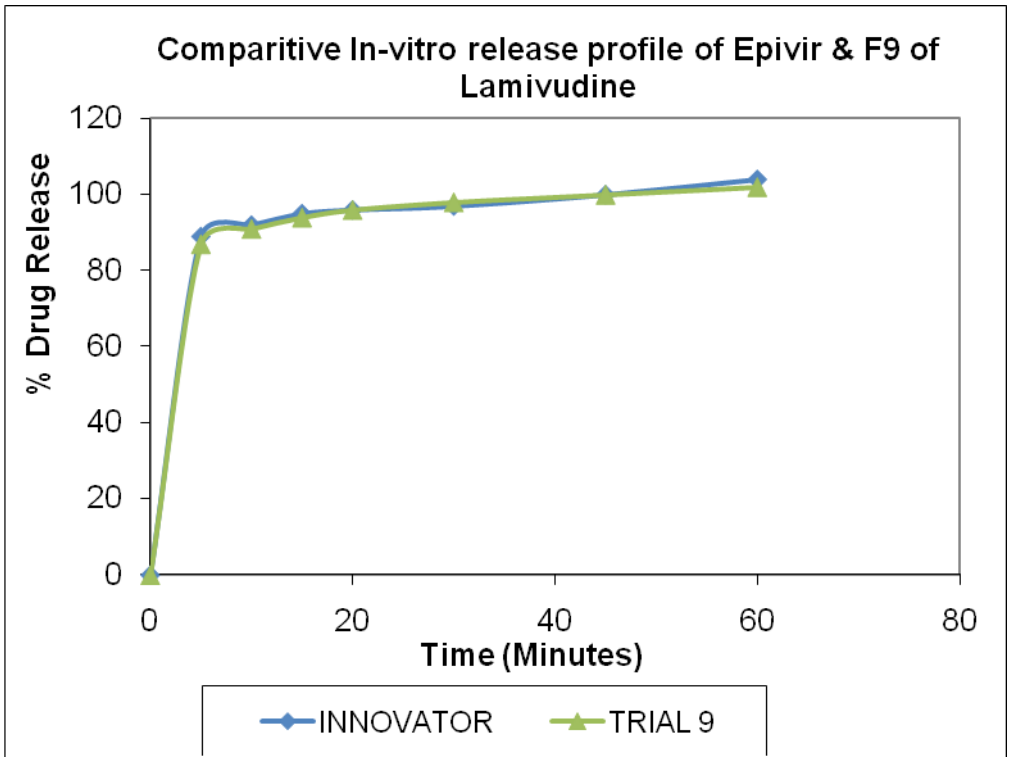


Fig.11.7.VIII In Vitro Release Profile of F9 with Epivir (Innovator)

11.8 The results are described as:

Based on the literature review, the immediate release tablet was prepared and the effect of superdisintegrant and method of formulation on drug release profile was observed.

The objective of the work was Formulation and evaluation of an immediate release tablets of the two anti retro-viral drugs

Formulation F1:

- In formulation F1 the two API's are mixed and done the dry granulation. The granules then compressed
- In formulation F1 CCS used as disintegrant, Magnesium stearate is used as lubricant,
- The formulation F1 having concentrations of CCS (7.0%), Magnesium stearate having concentration (2%)
- The percentage drug release from the tablets was found to be slow when compared with the innovator sample
- So it was decided to increase concentrations of CCS

Formulation F2:

- In this formulation F2 also the two API's was mixed and done the dry granulation. But in this the concentration of CCS was increased. Then these granules were compressed.
- The formulation F2 having concentration of CCS 14%, Magnesium stearate (2%)
- The formulation resulted in tablets in which the disintegration time was more and the percentage release was also found to be slow.
- So it was decided to change the method of formulation by separating the two API's and compacted separately and then mixed and compressed.

Formulation F3:

- In this formulation F3 the two API's compacted separately and then mixed and compressed.
- The formulation F3 having concentration CCS (4.0%) and SSG (5%) per tablet.
- The formulation didn't result in the improvement in dissolution profile of the API's.
- So it was decided to add extra granular materials after granulation.

Formulation F4:

- In this formulation F4 MCC (Avicel PH 102) and Magnesium Stearate was added as extragranular materials to the dried granules and mixed.
- In formulation F4 the concentration of CCS and SSG was same as that of F3.
- This formulation didn't show improvement of % drug release from the formulation.
- Hence it was decided to do wet granulation method instead of dry granulation.

Formulation F5:

- In this formulation F5 wet granulation method was used instead of dry granulation method
- The formulation F5 having concentration CCS (4.0%) and SSG (1.2%)
- This formulation resulted in slightly increased dissolution than the F4. The % release of Lamivudine was found to be satisfactory while % drug release from Tenofovir DF was very slow.
- So it was decided to increase the super disintegrant concentration inorder to enhance dissolution profile of Tenofovir DF from the formulation.

Formulation F6:

- In this formulation F6 wet granulation was used and the concentration of the super disintegrant was increased. Here SSG was also added to extragranular portion.
- The formulation F6 having concentration CCS (5%) and SSG (3%).
- The formulation F6 resulted in increase of Lamivudine % release but there is no improvement in the % release of Tenofovir DF when compared with the innovator product.
- So it was decided to change the method of formulation by separating the two API's and formulate bilayer tablets.

Formulation F7:

- In this formulation F7 the two API's are granulated by wet granulation method and the two granules are separated and compressed as bilayer tablets**.**
- The formulation F7 having concentration CCS (4%) and SSG (3%).
- The formulation F7 resulted in increase of dissolution profile of Lamivudine but the % release of Tenofovir DF from the formulation was slower than innovator product.
- So it was decided to increase the concentration of super disintegrants**.**

Formulation F8:

- In this formulation F8 the concentration of super disintegrants was increased than F7.
- The formulation F8 having concentration of CCS (6%) and SSG (5%).
- The formulation F8 resulted in increase of dissolution profile of Tenofovir DF and Lamivudine. The % release of Tenofovir DF and Lamivudine was slightly faster when compared to F7. But the % release of Tenofovir DF was slower than innovator product.
- So it was decided to increase the concentration of super disintegrants.

Formulation F9

- In this formulation F9 the concentration of super disintegrants was increased than F8.

- The formulation F9 having concentration of CCS (8%) and SSG (7%).
- The formulation F9 resulted in increase of dissolution profile of Tenofovir DF and Lamivudine and the % release from the formulation was comparable with the innovator product.
- So it was decided to take a trial to optimize F9 with similar formula as of F9 but reduce the size of Tenofovir DF by micronization. In order to optimize the formulation F9.

Formulation F10:

- In this formulation F10 the formula is similar to F9 but in this the trial the size of Tenofovir DFwas reduced by micronization process ie by passing through 80#.
- The formulation F10 having concentration of CCS (8%) and SSG (7%).
- The formulation F10 resulted in increase of dissolution profile of Tenofovir DF and Lamivudine and the % release of Tenofovir DF and Lamivudine was comparable with the innovator product but the % release from F9 was more comparable with Innovator.
- So it was decided to take a trial by increasing the concentration of MCC (Avicel PH 101) and decreasing the concentration of Pharmatose 200m

Formulation F11:

- In this formulation F11 the concentration of MCC (Avicel PH 101) is increased and the concentration of Pharmatose 200m is decreased.
- The formulation F11 having concentration of CCS (8%) and SSG (7%).
- The formulation F11 resulted in decrease of dissolution profile of two API's when compared with F9.

- So it was decided to take a trial by increasing the concentration of CCS and put the pregelatinized starch in the extra granular part.

Formulation F12:

- In this formulation F12 the concentration of CCS is increased and Pregelatinized starch was added in extra granular part.
- The formulation F12 having concentration of CCS (9%) and SSG (7%).
- The formulation F12 resulted in decrease of dissolution profile when compared with F9.
- So it was decided that optimized concentration of CCS is 8% and SSG is 7% and F9 is the optimized formula.

11.9 DATA ANALYSIS:

Table No: 11.9.1 Determination of order of release of Tenofovir DF & F9

Time (mins)	% Drug release	SQRT 't'	Log 't'	% drug remaining	log % drug remaining
0	0	0	0	0	0
5	72.48	2.23607	0.699	27.6	1.44
10	85.92	3.16228	1	14.08	1.14
15	88.88	3.87298	1.1761	11.12	1.046
20	93.12	4.47214	1.301	6.88	0.837
30	95.04	5.47723	1.4771	4.96	0.695
45	99.84	6.7082	1.6532	0.16	-0.7959
60	104.6	7.74597	1.7782	-4.65	-

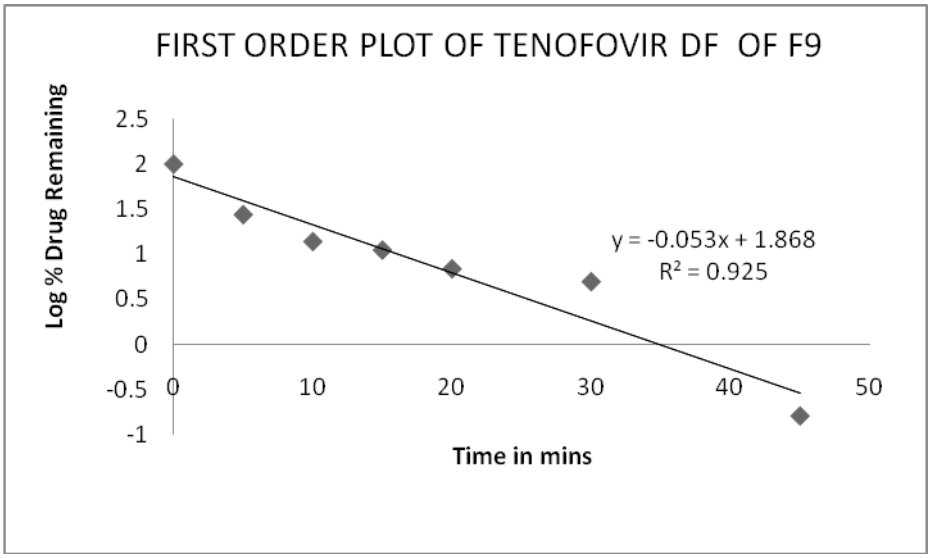


Fig.11.9.1 First order plot of Tenofovir DF

11.9.2 Drug Release Kinetics of Tenofovir DF, F9

Table No: 11.9.2 Drug Release Kinetics of Tenofovir DF, F9

Batch	Zero order		First order		Higuchi		Korsmeyer-Peppas	
	R^2	K_0 (mg/L/ hr)	r^2	K_1 (h^{-1})	r^2	K_{Hg} (h^{-1})	r^2	N
F9	0.320	0.929	0.925	-0.053	0.603	10.65	0.668	0.989

The curve fitting results of the release rate profile of the designed formulations gave an idea on the mechanism of drug release.

Based on the data analysis the drug release was found to follow First order release kinetics. This model indicates a coupling of the diffusion and erosion mechanism (Anomalous diffusion) and indicates that the drug release was controlled by more than one process.

Also, the drug release mechanism was best explained by first order, as the plots showed the highest linearity (r^2 = 0.925), as the drug release was best fitted in first order kinetics, it indicated that the rate of drug release takes place by diffusion and erosion mechanism and follow Anomalous diffusion (non-fickian, super case-II transfer).

Table No: 11.9.3 Determination of order of release of Lamivudine & F9

Time (mins)	% Drug release	SQRT 't'	Log 't'	% drug remaining	log % drug remaining
0	0	0	0	0	0
5	87.5	2.23607	0.699	12.5	1.0969
10	91.28	3.16228	1	8.72	0.9405
15	93.88	3.87298	1.1761	6.12	0.7868
20	94.8	4.47214	1.301	5.2	0.716
30	97.8	5.47723	1.4771	2.2	0.3424
45	99.84	6.7082	1.6532	0.16	-0.7959
60	102.11	7.74597	1.7782	-2.11	-

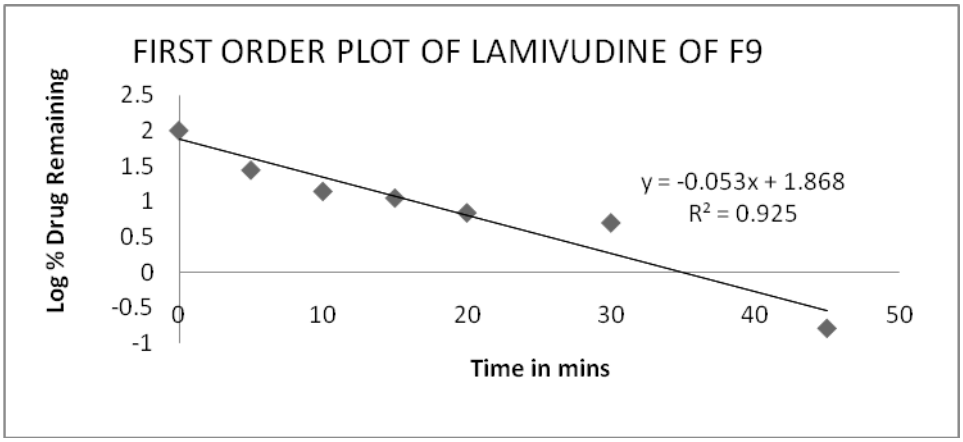


Fig.11.9.2 First order plot of Lamivudine

11.9.4 Drug Release Kinetics of Lamivudine, F9

Table No: 11.10.2. Drug Release Kinetics of Lamivudine, F9

Batch	Zero order		First order		Higuchi		Korsmeyer-Peppas	
	R^2	K_0 (mg/L/ hr)	r^2	K_1 (h^{-1})	r^2	K_{Hg} (h^{-1})	r^2	N
F9	0.439	1.081	0.925	-0.053	0.730	11.59	0.047	0.279

The curve fitting results of the release rate profile of the designed formulations gave an idea on the mechanism of drug release.

Based on the data analysis the drug release was found to follow First order release kinetics. This model indicates a coupling of the diffusion and erosion mechanism (Anomalous diffusion) and indicates that the drug release was controlled by more than one process.

Also, the drug release mechanism was best explained by first order, as the plots showed the highest linearity (r^2 = 0.925), as the drug release was best fitted in first order kinetics, it indicated

that the rate of drug release takes place by diffusion and erosion mechanism and follow Anomalous diffusion (non-fickian, super case-II transfer).

11.10. Similarity factor f2 and Dissimilarity factor f1

Table no: 11.10.1 Criteria for selection of optimized formulation of Tenofovir DF:

Time (hrs)	INNOVATOR of Tenofovir DF (R)	F9 (T)	/R-T/	/R-T/^{2}	f2 value	f1 value
0	0	0	0	0	83.8	1.04
5	74	72	2	4		
10	88	86	2	4		
15	91	89	2	4		
20	92	93	1	1		
30	94	95	1	1		
45	100	100	0	0		
60	106	105	1	1		
TOTAL	645	640	11	15		

The dissolution profiles of formulation F9 and innovator product of Tenofovir DF were compared by calculating similarity factor (f2) and dissimilarity factor (f1). The f2 and f1 was found to be 83.08 and 1.04 for the comparison of dissolution profiles of formulation F9 and innovator product. Hence these two products were considered to be similar.

Hence the above said formulations were selected for further evaluation.

Table no: 11.11.2 Criteria for selection of optimized formulation of Lamivudine:

Time (hrs)	INNOVATOR of Lamivudine	F9 (T)	/R-T/	/R-T/2	f2 value	f1 value

	(R)					
0	0	0	0	0	87.20	1.04
5	89	88	1	1		
10	92	91	1	1		
15	95	94	1	1		
20	96	95	1	1		
30	97	98	1	1		
45	100	100	0	0		
60	104	102	2	2		
TOTAL	673	668	7	9		

The dissolution profiles of formulation F9 and innovator product of Lamivudine were compared by calculating similarity factor (f2) and dissimilarity factor (f1). The f2 and f1 was found to be 87.20 and 1.04 for the comparison of dissolution profiles of formulation F9 and innovator product. Hence these two products were considered to be similar. Hence the above said formulations were selected for further evaluation

11.12. STABILITY STUDY OF IMMMEDIATE RELEASE BI-LAYER TABLET

Stability data of Film Coated Immediate Release Bilayer Tablet of Tenofovir DF and Lamivudine of formulation F9 are given below

Table no.11.12.1 Stability study of Bi-layer coated tablet of F9

Test	Specification	Initial	One month
Description	White colored, capsule shape biconvex film coated bilayered tablet with embossing "I" on one side and "49" on other side.	White colored, capsule shape biconvex film coated bilayered tablet with embossing "I" on one side and "49" on other side.	White colored, capsule shape biconvex film coated bilayered tablet with embossing "I" on one side and "49" on other side.
Dissolution: Medium=900ml 0.1N HCl, USP Apparatus II, 50 RPM, for 60min.	For Tenofovir DF	In 60 min = 104.86%	In 60 min = 102.96%
Dissolution: Medium=900ml, 0.1 N HCl, USP Apparatus I, 50 RPM, for 60min.	For Lamivudine	In 60 min = 102.86%	In 60 min = 101.74%
Assay	90 % to 110 % of stated amount of Tenofovir DF	Mean = 98.22%	Mean = 97.22%
	90 % to 110 % of stated amount of Lamivudine	Mean = 99.21%	Mean = 98.05%
Thickness	---	7.40-7.50mm	7.40-7.50mm
Hardness	---	22-26kp	23-26kp
Disintegration Time	---	1-3min	1-3 min

12. SUMMARY

Tenofovir DF and Lamivudine are nucleotide and nucleoside analog reverse transcriptase inhibitors and they are used in the treatment of AIDS & Hepatitis. The half-life of Tenofovir DF was 17 hours and Lamivudine was 7 hours. The daily dose of the two API's is 300 mg per day. So it was not that much required to formulate them in to sustained release formulation.The innovator samples of the two API's were in immediate release formulation.So it was formulated as immediate release formulation. But when the API's are formulated as combination tablets the percentage release was found to be very less. So it was formulated as bilayered tablets.

The daily dose of Tenofovir DF & Lamivudine was described as 300mg/300mg for only once daily.

Therefore an attempt is made to formulate immediate release formulation, which has disintegration time less than 15 min and the dissolution profile of the drug with very faster release.

In the formulation of immediate release tablet Croscarmellose sodium and Sodium starch glycolate –Type A was used as superdisintegrants, Pregelatinized starch used as binder. Other excipients used are lactose monohydrate (diluents) Microcrystalline Cellulose (diluents), Magnesium stearate (lubricating agent). Fourier transform Infrared spectroscopy confirmed the absence of any drug/polymers/excipients interactions.

The formulations F1, F2, F3 and F4 were prepared by dry granulation method, formulation F5 and F6 were prepared by wet granulation method and formulation F7 to F12 were formulated as bilayer tablets and the granules were prepared by wet granulation method.

Super disintegrants croscarmellose sodium and sodium starch glycolate were used to give immediate release for the tablets.

The tablets were compressed using 19.8× 10.2 mm Capsule shaped punch with embossing "I" on one side and "49" on other side and compressed on KAMBERT 8 station rotary compression machine and KARNAVATI 8 station rotatory bilayer machine.

The prepared immediate release tablets were evaluated for hardness, weight variation, thickness, friability, drug content uniformity, in-vitro dissolution studies. F9 formulation showed good evaluation studies and an immediate drug release.

Finally, Innovator obtained from the market and the dissolution profile of the final batch was matched with the innovator's dissolution profile.

From the dissolution profile, f1 & f2 value was calculated and from that value optimize batch of Tenofovir DF and Lamivudine was selected.

All formulations were subjected for four different models viz. Zero order, First order, Higuchi and Peppas model equations and the formulation best fit in to the First-order release kinetics that indicate the formulation had released the drug by concentration gradient. It was revealed that super disintegrants and method of formulation had significant influence on drug release.

Final Batch was charged for the stability study with packing at 40^0 C/75% RH for 1 month. The results obtained after the stability period was not having any change than the initial results.

Thus conclusion can be made that stable dosage form can be developed for Tenofovir DF & Lamivudine by bilayer method for immediate release by wet granulation.

13. CONCLUSION

The aim of the present study was to formulate and evaluate a stable immediate release tablet of Tenofovir DF and Lamivudine to maintain constant therapeutic levels of the drug and compare with the innovator samples. The immediate release bilayer tablets were prepared by wet granulation method with different concentrations of CCS & SSG Type-A as superdisintegrants.

The formulations F1, F2, F3 and F4 were prepared by dry granulation method. And the drug release of above formulations was slow. In all these formulations only 80% of the drug release was found within 60min for Tenofovir DF and only 90% drug release for Lamivudine within 60 min.

The formulation F5 and F6 were prepared by wet granulation method which resulted in increase in dissolution rate of Tenofovir DF and Lamivudine but it was not matching with innovator % drug release.

The formulation F7 to F12 were formulated as bilayer tablets and the granules were prepared by wet granulation method.

In the formulation F7 the concentration of superdisintegrants CCS was 4% and SSG was 3%. Here the drug release for Lamivudine was satisfactory but for Tenofovir DF it was slow.

In the formulation F8 the concentration of super disintegrants CCS was 6% and SSG was 5%. Here also drug release for Lamivudine was satisfactory but for Tenofovir DF it was slow.

In the formulation F9 the concentration of super disintegrants was CCS 8% and SSG was 7%. Here drug release of both Tenofovir DF and Lamivudine was comparable with innovator on the basis of similarity and dissimilarity factors.

The formulations F10, F11 and F12 were done to optimize the F9.In the formulation F10 the concentration of super disintegrants was CCS 8% and SSG 7%. Here the Tenofovir DF drug was micronized to reduce the particle size. Then the drug release of both Tenofovir DF and Lamivudine was comparable with innovators but F9 was more comparable with innovator.

In F11 the concentration of Lactose monohydrate (pharmatose 200m) and microcrystalline cellulose (Avicel PH 101) was changed. In F12 the concentration of CCS increased and binder Pregelatinized starch added as extragranular part of Tenofovir DF.

The results of dissolution studies indicated that formulation F9 is the most successful of the study, exhibited drug release pattern close to innovator drug release profile. The drug content of F9 was found to be better than other formulation, so that it was selected as optimized formulation. The designed immediate release bilayered tablets of formulation F9 release 85.92% of Tenofovir DF in 10 min and 104.65% at 60min and 91.29% of Lamivudine in 10 min and 102.11% at 60min. Regulated drug release in First order kinetics attained with this formulation, as the highest linearity (r^2 = 0.925) showed with first order plot.

The developed immediate release bilayer tablet formulation was quite stable with regard to drug content, physical properties and dissolution rate.

Hence it can be concluded that once daily immediate release bilayer tablet of Tenofovir DF and Lamivudine having satisfactory drug release profile which may provide an increased therapeutic efficacy.

1. 14. Bibliography:

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